Glucose/Galactose Binding Protein (GBP) is a member of the periplasmic binding protein family, and is natively expressed by Escherichia coli. The structure of this binding protein consists of two globular domains connected by a short hinge region. Importantly, as its name implies, GBP is a naturally selective probe for glucose, and contains a binding site for this small molecule between its two globular domains. In the presence of glucose, these two domains close together around the glucose molecule, dramatically altering the conformation of the protein. Within this centrally located binding pocket are one of the five tryptophan (TRP) residues, and one of the seven phenylalanine (PHE) residues. We have previously shown that through the incorporation of non-natural amino acids (NNAA), it is possible to confer unique properties to the target protein. Specifically, we have previously developed glucose sensors possessing unique fluorescence characteristics through incorporation of the NNAAs 7-azatryptophan and 5-fluorotryptophan. In the current work, we demonstrate the successful global incorporation of 3,4-dihydroxyphenylalanin (L-DOPA) into GBP in the place of PHE. L-DOPA contains a catechol moiety that participates in a quasi-reversible, 2-electron redox process. The conformational change that occurs as a result of substrate binding buries the amino acid residues surrounding the binding pocket and alters their local environment. We demonstrate that this conformational change upon substrate binding is detectable as a change in the electrochemical behavior of incorporated L-DOPA residues, and that this newly synthesized electroactive GBP biosensor can be used for the selective electrochemical detection of glucose. To the best of our knowledge, this represents the first report of electrochemical detection of glucose via inherently electroactive amino acids incorporated into the primary sequence of a protein.

Keywords: Amino Acids, Bioanalytical, Biosensors, Electrochemistry
Application Code: Bioanalytical
Methodology Code: Electrochemistry
Artemisinin is a sesquiterpene lactone and a popular malaria drug. Understanding the mechanism of action of this drug is important, given recent reports of emergence of resistance of malaria to the drug. We are investigating the biocatalytic reduction of ART on hemoglobin anchored on carbon nanofibers using electrochemistry. The drug interaction with hemoglobin in free solution using liquid chromatography and UV-visible spectroscopy is also being studied. Our results show a linear relationship between the catalytic peak current and the artemisinin concentration. The current linear range obtained was from 0 to 200 mM and with 0.329 mA/mM sensitivity. The calculated apparent Michaelis–Menten constant Km for hemoglobin anchored on carbon nanofibers towards catalysis of ART was 0.093 mM. The results of analysis of free hemoglobin reacted with artemisinin monitored using UV-visible spectroscopy and liquid chromatography will be discussed.

Keywords: Bioanalytical, Biosensors, Chromatography, Electrochemistry
Application Code: Bioanalytical
Methodology Code: Sensors
DNA detection is important to many biological processes. DNA biosensors are increasingly used in hybridization reactions, mutation detection, genomic sequencing, and identification of pathogens. The macromolecular polysaccharide-based polyanions, including heparin salts and carrageenan have unique properties and functions in physiology, and food technology. The quantity of polyanion reflecting the exact number of charges in samples administered in biological procedures has to be strictly controlled. Thus the detection of these polyanions in clinical or commercial samples is key in the diagnostic and quality control processes of related fields.

Novel Os(II) carbonyl complexes with two phenanthroline and a 4-phenyl pyridine, imidazole or phenyl imidazole group exhibit moderate emission intensity in the visible region. Our recent results show that the luminescence intensity of these new osmium complexes can be significantly reduced or enhanced by different DNA strands. This work presents the UV/vis absorbance and luminescence responses of the Os(II) complexes to various DNA samples from natural sources and synthetic; as well as other polyanions The binding constants are calculated with the luminescence and absorbance responses. These studied will provide information in the effort of developing DNA detection markers and/or binding agents for biological applications.

Sensing of oxyanions such as phosphates and carbolylates are very important because of their vital roles in essential biological processes. Our work also studied the responses of Osmium(II) complexes to small anions such as dihydrogen phosphate and acetate anions and the results will be presented.

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

**Application Code:** Bioanalytical

**Methodology Code:** Sensors
Designing high performance functional materials often requires a deep understanding of the fundamental relationships between physical and chemical properties. For measurements such as these, bulk sampling methods are typically sufficient; however, for the development of thin films and coatings, it is usually necessary to investigate these relationships across a surface, demanding advanced imaging techniques. Over the past few years, research from our group has focused on the development and application of novel spectroscopic imaging methods to elucidate the relationship between various chemical and physical properties of advanced and hybrid functional materials across length scales. This presentation includes various research projects that best represent this work. The first project presented includes the application of statistical methods with colocalized Raman and scanning probe microscopy to study a binary and ternary hybrid xerogel thin films with antifouling properties. The second project shows the application of the same techniques for the analysis of 2D MoS2 nanomaterials. The third presents a study of 2D WS2 with tip-enhanced Raman spectroscopy (TERS). Finally, future work on the development of additional spectroscopic imaging methods from my current lab will be presented.

Keywords: Imaging, Material Science, Spectroscopy, Surface Analysis
Application Code: Material Science
Methodology Code: Surface Analysis/Imaging
We present the synthesis of two peptide-based chiral ionic liquids (CILs) derived from a Glycine-L-histidine cation and two perfluorinated anions; bis(trifluoroethyl)sulfonyl imide and bis(pentafluoro ethanesulfonyl) imide anions. The CILs were synthesized via ion-exchange and were evaluated for their purity, melting points, thermal stability, and chiral recognition ability. Thermogravimetric analysis (TGA) was performed and it was determined that the thermal decomposition temperature of the Gly-His bis(trifluoroethyl) sulfonyl imide IL was between 300 and 350 degrees Celsius, and for the Gly-His bis(pentafluoro ethanesulfonyl) imide the thermal decomposition temperature was determined to be 384.70 degrees Celsius. Differential scanning calorimetry (DSC) was performed and the melting point of the Gly-His bis(trifluoroethyl) sulfonyl imide IL was found to be close to 70 degrees Celsius and the Gly-His bis(pentafluoro ethanesulfonyl) imide IL had a melting point close to 40 degrees Celsius. Based on fluorescence spectroscopy studies, it was determined that the Gly-His bis(trifluoroethyl) sulfonyl imide ionic liquid exhibited chiral recognition ability using naproxen, propranolol, and TFAE as the chiral analytes. The Gly-His bis(trifluoroethyl) sulfonyl imide IL effectively served as a chiral selector. Studies of the chiral recognition ability of Gly-His bis(pentafluoro ethanesulfonyl) imide IL are underway for the same chiral analytes. The high thermal stability of these CILs indicate they have potential to be used as chiral selectors in high temperature reactions and as stationary phases for gas chromatography (GC).
Global transcriptomic investigation of the human macrophage response to infection with mycobacteria. To enumerate, compare (RNAseq) and validate (qPCR) differentially expressed transcriptomes from MDM’s after stimulation with various mycobacterial strains and to knock-down (siRNA)/knock-up (saRNA) of selected differentially expressed mRNA. Tuberculosis is one of the biggest infectious diseases causing millions of deaths every year around the globe. Mycobacterium tuberculosis (M.tb) is the causative agent of this disease. Macrophages are the primary line of host immune defense against the invasion of this bacterium. Infection in humans is decided by the efficacy of macrophages either to contain or successfully eliminate different bacterial strains. For example, M. smegmatis survives up to 12 hours inside a macrophage, while many pathogenic strains can survive longer. The study of macrophage response to infection with M.tb can provide a host signature responsible for the intra-cellular survival of this bacterium. Previously published studies on the interaction of host immune response and M.tb; have used Mycobacteria grown on media having detergents, like tween to avoid clumping of cells. Tween alters the composition of the M.tb cell wall and decreases bacterial virulence. Hence, In the proposed study, we are trying to mimic the ex-vivo environment for M.tb. We will isolate PBMCs from healthy study participants followed by their maturation to macrophages and subsequent infection with pathogenic and non-pathogenic mycobacteria. We will extract total RNA after 12 and 96 hours of infection. Total RNA will be subjected to Ampliseq and results will be validated by qPCR. Differentially expressed genes will be knocked-down/up using si/saRNA for exploring the novel therapeutic targets. Post-infection supernatants at different interval will be used for studying dynamics of various cytokines during infection.

Keywords: Biotechnology, Drug Discovery, Drugs, Gene Therapy
Application Code: Drug Discovery
Methodology Code: New Method
An ever-growing number of synthetic cathinones (bath salts) are manufactured as illicit drugs and sometimes marketed as plant food or insect repellent to avoid prosecution. Methylone, mephedrone, and methylenedioxypyrovalerone (MDPV) are the most common components of these designer drugs. The increased prevalence of bath salt usage has led to the need to detect and quantify these hallucinogens. Whereas existing LC-MS methods have been established for these drugs, these methods are not routinely employed and can add unnecessary time and cost to routine drug screenings. Capillary transient isotachophoresis (ctITP) coupled with laser induced fluorescence (LIF) detection can be used to separate and detect fluorescently labeled bath salts in patient samples in a more cost and time efficient manner. Whereas only some synthetic cathinones are natively fluorescent, all can be rendered fluorescent upon derivatization with carefully chosen fluorescent probes. In particular, we will focus on novel squarylium dyes functionalized with 4-sulfo-2,3,5,6-tetrafluorophenyl ester (STP ester) to facilitate covalent derivatization of the amine-based drug targets. Squarylium dyes possess attractive qualities including ease of functionalization, high molar absorptivities, narrow absorption and emission bands, and enhanced emission intensities upon analyte binding. Fluorescent determination of synthetic cathinones employing SQ-STP dyes will be compared to commercially available probes, and furthermore, the application of multiple discriminate analysis (MDA) to aid in the identification of synthetic cathinones in routine sensing protocols will be explored. The ultimate goal of this work is to be able to apply the SQ-STP dyes as on-column labels for quantitative ctITP determinations of bath salts in patient-positive samples.

Keywords: Capillary Electrophoresis, Clinical/Toxicology, Drugs, Fluorescence
Application Code: Clinical/Toxicology
Methodology Code: Capillary Electrophoresis
Capillary electrophoresis (CE) is an effective and powerful technique for aptamer selection against targets of interest ranging from the microbial to the molecular. However, aptamer selection against small molecule targets remains challenging for a number of reasons, such as weaker binding and minimal perturbation of aptamer mobility upon small molecule target binding. The potential to use carbon-based nanomaterials such as carbon dots (CDs) to mediate the mobility and binding properties of single-stranded DNA (ssDNA) aptamers is thus of interest. The goal of the current study is to characterize the nature of interactions between CDs and ssDNA aptamers, and to utilize such interactions to optimize the CE separation of free and target-bound ssDNA aptamers.

Carbon dots were synthesized by a ‘bottom-up’ approach involving the carbonization of citric acid or other simple organic precursor. The CDs (with [wavelength][sub]ex[/sub] and [wavelength][sub]em[/sub] ~ 375 and 450 nm, respectively) were used at a 1:10 dilution yielding an approximate concentration of 0.5 mg/mL when mixed with 500 nM ssDNA samples. Preliminary results show that interaction of ssDNA and CDs depends both on buffer pH and incubation time. Interactions are studied by monitoring changes in fluorescence emission of the CDs themselves, or of the ssDNA or ssDNA-CD complexes. Emission of ssDNA and its complexes was monitored at 520 nm by incorporation of the fluorescent intercalating reagent SYBR Gold ([wavelength][sub]ex[/sub] = 488 nm) in buffer. Additionally, changes in mobility of ssDNA can be used to study interactions with CDs. To this end, we employed a modified capillary transient isotachophoresis (ctITP) method, with a tris-glycine separation buffer and a tris-HCl sample buffer. Ultimately, when target is introduced to the ssDNA–CD mixture, a competition for aptamer binding sites will occur, thus resulting in a unique peak for the target-bound aptamer.

Keywords: Bioanalytical, Capillary Electrophoresis, Fluorescence, Optimization
Application Code: Bioanalytical
Methodology Code: Capillary Electrophoresis
GC-MS with Cold EI is based on GC and MS interface with supersonic molecular beams (SMB) and electron ionization (EI) of cold compounds in the SMB (hence the name Cold EI). The GC eluting compounds are mixed with helium, expand from a supersonic nozzle into a vacuum chamber, vibrationally cooled, skimmed, collimated into a SMB, are ionized in a contact-free fly-through EI ion source and are mass analyzed by a single quadruple MS. GC-MS with Cold EI extends the boundaries of GC-MS capabilities via the provision of enhanced molecular ions, improved sample identification, significantly extended range of compounds amenable for analysis, much faster analysis, lower limits of detection particularly for difficult to analyze compound, uniform response, very low vacuum background, ultra-fast ion source response time and no ion source peak tailing thus improved separation.

The poster will list 25 improved capabilities some of which are unexpected benefits such as:
A) NIST library identification probabilities are improved with Cold EI as the molecular ions are the most sample characteristic ions.
B) Doubly charged molecular ions are exhibited for large molecules, thus doubling the analysis mass range.
C) Under 2 minutes analysis time is uniquely achieved with column flow programming since the ion source response is independent on the column flow rate.
D) The selectivity against matrix interference on the Cold EI molecular ion is as effective as MS-MS on a fragment ion.
E) Cold EI is more effectively coupled with the TAMI software to provide elemental formula for improved identification.
Explosives are a class of xenobiotics, which pose a permanent and increasing concern on human health and ecosystem. These chemicals are highly toxic, some carcinogenic, and their detection in areas surrounding military bases and weapon training facilities became imperative. Quality of potable water, particularly in rural areas where ground wells are the primary water sources is of great importance. The ultimate scope of measuring these environmental contaminants is to build effective strategies for site protection, remediation and removal. Current work focuses on presenting chromatographic separation/mass spectrometric analysis of selected explosives. Successful identification with specific mass transitions and characteristic fragment assignments is presented for target nitroaromatics, nitroesters and nitroamines.

Keywords: Forensic Chemistry
Application Code: Environmental
Methodology Code: Liquid Chromatography/Mass Spectrometry
An environmental analysis of the Genesee River was carried out at four sites near Rochester NY in order to determine the concentration of phosphorus, nitrates and triclosan. Triclosan (5-chloro-2-(2,4-dichlorophenoxy)phenol) is an antimicrobial agent used in a wide array of personal care and consumer products that eventually get in our waterways. A reversed phase solid-phase extraction (SPE) method was developed and optimized for extraction of triclosan in the Genesee River water. A reverse phased HPLC method based on a C18 column, 280 nm detection and 75:25 acetonitrile/water mobile phase was developed and optimized for determination of triclosan in the Genesee River water. The HPLC method was validated using standard addition calibration method in order to determine the concentration of triclosan, detection and quantification limits, precision and recovery. UV-Visible spectrophotometry utilizing external standard calibration was employed for the determination of the concentrations of phosphates and nitrates. Triclosan was determined to be present at concentrations that ranged from 0.0368 to 0.235 ppm. The concentration of phosphorus and nitrates ranged from 0.02 to 4.18 ppm and were within the acceptable levels for drinking water. This community based environmental analysis of the Genesee River presents a good opportunity for the investigators to face a real world situation.

Keywords: Environmental/Water, HPLC, Solid Phase Extraction, UV-VIS Absorbance/Luminescence
Application Code: Environmental
Methodology Code: Liquid Chromatography
Abstract Text

Mass spectrometry is a versatile, sensitive tool that covers a vast array of applications including environmental, forensics, clinical, proteomics, drug metabolism and quality control to name a few. This diversity presents tremendous challenges to automating repetitive analytical procedures and the varying needs of each laboratory. While instrument vendors provide a vast number of different software packages to address the most common applications, they are typically limited in their scope and unfortunately, are proprietary to each vendor platform. This also makes it challenging to integrate third party applications which further restricts users from implement efficient workflows.

In this poster, we will discuss a new approach to enable a vendor independent implementation of workflows using a novel programmable toolbox. The toolbox leverages the widely used open source Python programming language whose high-level attributes and extensive support in the scientific community provide a near ideal environment for automating processes and integrating third party analysis routines. Key to the software is a set of extensible software modules with the ability to directly read most MS vendor formats for vendor independent processing.

Applications of the toolbox will be demonstrated including: Processing and analysis of GC/MS data with automatic identification of unresolved chromatographic peaks; rapid screening and quantitation of pharmaceutical impurities in biologics; and the rapid identification of materials using direct ionization techniques on low resolution quadrupoles.

Keywords: Automation, Laboratory Informatics, Mass Spectrometry
Application Code: General Interest
Methodology Code: Mass Spectrometry
Perfluoroalkyl substances (PFASs) are highly fluorinated alkyl chemicals where one or more H substituents have been replaced by F. Surfactants and polymers comprised of PFASs have been used in industrial and commercial applications such as fire fighting foams. Analysis of PFASs is important for environmental monitoring. Microporous organosilica materials have been developed to adsorb a broad range of PFASs including PFOA and PFOS, but also fluorinated substances with cationic and zwitterionic groups. The approach is to use self-assembly of alkoxysilane precursors to create pore structures possessing mixed mode surface chemistry including fluoroalkyl groups. Application of these materials for solid-phase extraction and downstream analysis by LC-MS will be discussed.
There is an ongoing need for an accurate, affordable, and simple point-of-care test to detect Bacterial Vaginosis (BV). BV is characterized as a change from a Lactobacillus-dominant vaginal microbiota to an anaerobic and facultative bacterial dominance, leaving patients with vaginal discharge, vaginal malodor, and extreme discomfort. According to the Center of Disease Control, “BV is the most common cause of vaginal symptoms among women...the prevalence in the US is estimated to be 21.2 million (29.2 %) among women ages 14 to 49. BV negatively affects patient’s quality of life, increases the risks of acquiring a sexually transmitted infection as well as the likelihood of miscarriages. Currently, the diagnosis of BV is quite inaccurate and complex, leading to high rates of misdiagnosis. Our project involves designing a diagnostic test for the presence of Gardnerella vaginalis. Because there are 17 different strains of Gardnerella vaginalis, the toxin vaginolysin that is secreted by all Gardnerella vaginalis strains is detected. We have developed a method for the expression, purification, and detection of vaginolysin via an ELISA, which, ultimately, will be translated into a point-of-care device by adapting it into a lateral flow assay. This study will be expanded to include the detection of Lactobacillus, Mobiluncus, Prevotella, Bacteroides, Peptostreptococcus, and the seven other bacteria that leave the vagina in an unbalanced state, leading to a point of care test to detect bacterial vaginosis.

Keywords: Biotechnology, Immunoassay, UV-VIS Absorbance/Luminescence
Application Code: Bioanalytical
Methodology Code: UV/VIS
### Abstract Text

Adenine nucleotides play an important role in biological systems since they serve as units for DNA and RNA synthesis and allosteric regulators of enzymes. Capillary electrophoresis is particularly powerful and well-suited for analyzing adenine nucleotides levels for its high separation efficiency and rapid analysis, inexpensive buffer salts, and smaller quantities of both buffer and sample. One way to improve the sensitivity of CE with UV detection without any chemical derivatization is to use an on-column preconcentration technique. Of the on-capillary stacking methods, isotachophoresis (ITP) is especially suitable for high-salt samples. Of the various compounds tested for leading electrolytes (LE), *alanine showed the best signal enhancement. The CE separation was performed on the Beckman P/ACE MDQ system with a photodiode array detector. All the analyses were performed at ambient temperature. The best separation were obtained in 100 mM Tris buffer (pH=7), 0.5 mM TTAC and 15% acetonitrile with 25 µm i.d. x 63.5 cm (total length) fused silica capillary at field strength of -433 V/cm. At optimum ITP conditions ATP, ADP and AMP could be detected at 10 µM, 10 µM and 12.5 µM concentrations respectively in 10 min.

Microchip electrophoresis is a versatile separation technique that can be used to analyzing of adenine nucleotides. For a more useful and universal detection of charged species, capacitively coupled contactless conductivity detection (C4D) was used. A BK Precision function generator was used to apply a signal of 400-500 kHz (optimized daily) with an amplitude of 10 Vpp at the working electrode. The detection circuitry was fabricated in-house. The voltage output was collected and converted from analog to digital through an in-house Labview program. Progress toward the use of this detection method for the determination of adenine nucleotides in biological samples will be discussed.

### Keywords
- Bioanalytical
- Capillary Electrophoresis
- Electrophoresis
- Lab-on-a-Chip/Microfluidics

### Application Code
- Bioanalytical

### Methodology Code
- Capillary Electrophoresis
Capillary electrophoresis (CE) has become a valuable tool for analyzing small molecules associated with metabolism and neurochemistry. CE provides a complementary separation to liquid chromatography, and has advantages in its speed, separation efficiency, and compatibility with small samples. An important advantage of CE is the ability to easily implement online preconcentration techniques, greatly improving sensitivity. Using the preconcentration technique - electrokinetic supercharging (EKS) - a method has been developed which allows for neurochemical monitoring using CE with UV-Vis detection. With this method, quantification of multiple neurotransmitters in brain tissue extract was achieved in a sixteen-minute separation (Figure 1). However, UV-Vis detection lacks selectivity, making it unsuitable for analyzing multiple analytes in complex samples.

CE coupled to mass spectrometry (MS) is a quickly rising tool for neurochemistry. We have transferred methodology from the CE-EKS-UV method to combine EKS with CE-ESI-MS to provide a high sensitivity, high selectivity method for analysis of neurotransmitters. This preconcentration technology improves sensitivity 4,500-fold as compared to a conventional separation, and has a separation time under six minutes for the four tested neurotransmitters. The developed method enabled detection of target analytes in complicated biological matrices such as dialysate and urine with no sample pretreatment. This method provides an LOD’s of 71-125pM for samples in low ionic strength matrices (i.e. tissue extract) and 71-125nM for samples in high ionic strength matrices (i.e. dialysate). The sensitivity, selectivity, and minimal sample requirements of this method make it suitable for monitoring neurotransmitters and metabolites in vivo, and has many applications including the study of disease, behavior, and drug effects.

Keywords: Capillary Electrophoresis, Electrospray, Quadrupole MS, Separation Sciences
Application Code: Bioanalytical
Methodology Code: Capillary Electrophoresis
**Abstract Text**

3D printing is emerging as a valuable tool in the development of microanalytical instrumentation. Most consumer grade 3D printers do not yet offer sufficient resolution to print precision microdevices, but they can be utilized in the development of integrated microanalytical systems while achieving substantial reductions in cost and development time. Here we describe the design and optimization of a low cost, miniature 3D printed LED-induced fluorescence (LED-IF) detection system for use in capillary electrophoresis. LED-IF offers reduced cost when compared to laser-induced fluorescence, and in many cases can achieve similar detection limits. Surface mounted LEDs and other miniature optical components enabled the LED-IF detection head to be packaged in a volume smaller than 1 cubic inch. Within the miniature detection head, light from a high intensity 490 nm LED was passed through an excitation bandpass filter and focused onto the capillary detection window by a spherical lens. A second spherical lens collected fluorescent emission at 90° from the excitation axis and transmitted it to a multimode fiber bundle for photon detection outside of the detection head. The miniature design of the optical system facilitated straightforward integration with commercial capillary electrophoresis instruments equipped with only UV-Visible absorbance diode array detectors. Factors including device geometry, excitation intensity, and detector gain were optimized, and detection limits on the order of 3 nM fluorescein in borate (pH 8) have been achieved. The application of this detection system to capillary zone and capillary gel electrophoresis separations of biomolecules will also be discussed.

**Keywords:** Capillary Electrophoresis, Detection, Fluorescence

**Application Code:** General Interest

**Methodology Code:** Capillary Electrophoresis
Capillary electrochromatography (CEC) is a hybrid technique integrated with advantages of capillary electrophoresis (CE) and liquid chromatography (LC). It has high separation selectivity in micro-LC, high performance in CE and low consumption of sample and mobile phase. It is a hot topic in CEC to develop new stationary phase for meeting the requirement of exceptional separation selectivity in pharmaceutical analysis by using new molecular recognition materials.

Metal organic frameworks (MOFs) and covalent organic frameworks (COFs) are a new family of molecular recognition materials. The development of MOFs and COFs as CEC stationary phase in CEC is a challenging work. Mussels attach to solid surfaces such as rocks in the sea. The tight adhesion results from the adhesive protein containing polydopamine secreted by mussels. Inspired by the composition of adhesive proteins in mussels and combined with the chemical modification of MOFs and COFs on the surface of capillary, we have successfully developed several novel capillary electrochromatographic columns [1-8] and applied in environmental and pharmaceutical analysis.

This talk will introduce the preparation, characterization, validation, and application of novel open tubular capillary electrochromatographic columns, based on mussel-inspired polydopamine functionalization and growth of MOFs and COFs in the inner wall of fused silica capillary using liquid-phase epitaxy. Keywords: Capillary electrochromatography, Polydopamine functionalization, Metal organic frameworks, Covalent organic framework, Pharmaceutical analysis.

Reference:

Keywords: Capillary Electrophoresis, Pharmaceutical
Metabolomics studies have revealed aberrant amino acid metabolism in breast cancer cell models that have been supported by recent findings of altered levels of amino acid derivatives in the urine of women diagnosed with breast cancer. A method for separating and quantifying the proposed amino acid panel by the use of capillary electrophoresis has been validated using spiked recovery analyses to determine method accuracy and reproducibility, and linear regression analyses to determine method detection limits, linearity, and linear range. Finally, the validated method was applied to the characterization of 150 urine samples collected from women with newly diagnosed breast cancer and healthy controls. In summary, this method has the potential to be used to support targeted studies of amino acid derivatives in epidemiological populations to better understand the significance of amino acid metabolism in cancer and its application to early breast cancer detection. The detailed method development and results of this study, such as method detection limit and quantitation limit, correlation of levels of individual amino acids in urine samples with the stages of breast cancer that were obtained from clinical pathology, will be presented at the conference.

Keywords: Amino Acids, Bioanalytical, Biomedical, Capillary Electrophoresis

Application Code: Biomedical

Methodology Code: Capillary Electrophoresis
MicroRNAs (miRNAs) are involved in many biological pathways and can serve as important biomarkers of disease. Most methods for miRNA detection require lengthy sample preparation and amplification steps that can bias the results. In addition, lack of specificity and reproducibility give rise to various challenges in detection of circulating miRNAs in biological samples. In this work, we applied the Single Molecule Array (Simoa) technique to develop an ultra-sensitive sandwich assay for direct detection of multiple miRNAs without pre-amplification. We successfully detected miRNAs at femtomolar concentrations with detection limits ranging from 1 to 30 fM, and high specificity by distinguishing miRNAs with a single nucleotide mismatch. This method was effective against a range of diverse target sequences, suggesting a general approach for miRNA detection. To demonstrate the practical application of this technique, we detected miRNAs in a variety of sample types including human serum and total RNA. The high sensitivity and simple workflow of the Simoa method represent excellent advantages for miRNA-based diagnostics of human diseases.

Keywords: Biological Samples, Biosensors, Biotechnology, Nucleic Acids
Application Code: Bioanalytical
Methodology Code: Sensors
Fluorophores originating in living organisms have long inspired diverse applications of biofluorescent material. Yellow-emitting berberine from tree turmeric was used anciently in Chinese folk medicine but more recently has found application in histology staining; sweet-smelling coumarin from tonka beans was isolated in the 19th century for perfumes but is currently employed as the synthetic starting material for customizable gain medium in dye lasers. Utilizing organisms as sources of fluorophores relieves the demand for petroleum feedstock in organic synthesis of fluorescent products. Endophytic fungi provide a promising vein for identification and isolation of natural fluorescent products and are the focus of this research. We report an endophytic fungus isolated from Carya aquatic (water hickory) showing endogenous fluorescence that was measured in the live organism, and analysis of the extracted material shows pH-dependent spectral properties. Interpretation of the spectra was accomplished through the use of time-dependent density functional-theory methods. The combined use of experimental and theoretically predicted spectra revealed the pH equilibria and tautomerization of the natural product, 5-methylmellein. This product shows promise both as a stand-alone pH-indicating fluorophore with alkaline pKa, and as "green" feedstock for synthesis of custom fluorophores.

Keywords: Biospectroscopy, Fluorescence, Microscopy
Application Code: Bioanalytical
Methodology Code: Fluorescence/Luminescence
The specificity of DNA-lipid micelles can be increased by the introduction of an aptamer. However, the micelle structure would be destabilized when interacting with cell membranes under certain conditions and therefore lose its selectivity for target cancer cells. Therefore, we herein report a straightforward cross-linking strategy to equip aptamer-lipid micelles with enhanced stability and improved specificity to target cancer cells. This facile design incorporates a methacrylamide branch to link aptamer and lipid segments. DNA-methacrylamide-lipid unit can be cross-linked via an efficient free-radical polymerization process under UV light after self-assembly in aqueous solution. In contrast to traditional non-cross-linked DNA micelles, by examining moving mobility using SDS-PAGE and evaluating spherical structure using DLS and TEM, covalently linked aptamer-lipid units have been shown to help reinforce the micelle structure. Then, after performing serum stability study and DNase I resistance study, we have demonstrated the enhanced aptamer probe stability of cross-linked micelles. Finally, the improved targeting ability of the resulting cross-linked nanoassembly was evidenced by both flow cytometry and confocal microscopy. In addition to developing a facile cross-linking method, this work clarifies the relationship between aptamer-lipid concentrations and the corresponding binding abilities. Moreover, the excellent biostability and biocompatibility of cross-linked aptamer-lipid micelles could facilitate their applications in more complex biological systems, such as biomedicine, biosensing and bioimaging.
Fluorescent proteins (FPs) have emerged as valuable biological markers for gene expression and protein tracking in living systems. Developing synthetic materials mimicking natural fluorescent proteins is crucial for elucidating photo-physical mechanism of FPs and creating new emission-responsive materials and biosensors. Molecular engineering of FPs and their mimics is an efficient approach to provide new bio-molecular toolkits for biotechnology and bio-analytical chemistry. Herein, we reported a series of new bio-sensing and bio-imaging approaches based on engineered FPs and their molecular mimics. 1) Using supercharged GFP (ScGFP) as the signal reporter, a homogenous method for DNA detection was developed based on the polyionic complex of ScGFP/DNA and toehold strand displacement. This assay was further applied to analyze site-specific DNA methylation status of human colon carcinoma tissue samples. 2) Label-free and homogenous analysis of base excision repair enzymes was achieved based on DNA-mediated ScGFP/GO interaction. 3) A convenient strategy for probing PARP-1 activity was proposed based on FRET between cationic conjugated polymer and ScGFP. 4) A biosensor was developed for Cu$^{2+}$ sensing relied on the multivalent metal ion-binding property of H[39]GFP and fluorescence quenching effect of Cu$^{2+}$. 5) Based on metal ion-mediated self-assembly of H[39]GFP, a protein delivery nano-system was developed for tumor-targeting protein release. 6) Based stimuli-dependent surface charge tuneable property of HisGFP, we presented an intelligent delivery carrier for high efficiency transfection of functional nucleic acids and proteins. 7) We developed a set of DNA mimics of RFPs with high quantum yields, large Stokes shifts, excellent anti-photobleaching properties, and two-photon fluorescence. The DNA RFP mimics have been exploited for membrane protein imaging in live cells and tissue samples without the need for genetic coding.

**Keywords:** Bioanalytical, Biosensors, Imaging, Protein

**Application Code:** Bioanalytical

**Methodology Code:** Fluorescence/Luminescence
Introduction and Results: While numerous signal amplification techniques have been developed for the detection of low levels of biological species, manipulating them in living cells is still challenging due to the requirement of rigorous reaction conditions and utilization of exotic enzymes or other biofunctional materials as auxiliary amplification units. Herein, we set out to propose a new signal amplification concept, called cell-assisted in situ fluorescence amplification (CAFA), amenable to successfully address this issue. The key innovation of CAFA is taking the intracellular matrixes, rather than exotic materials as the signal amplification components to operate the amplified analysis (Fig.1). To establish a prototype of CAFA, we have developed an intracellular proteins-assisted fluorescence amplifier (OH-CA) enables us to track basal hydroxyl radicals ($\cdot$OH) levels and fluctuation in living cells. In our proposed approach, OH-CA comprises functional nano-container, $\cdot$OH-trigger antenna, signal regulator, and amounts of protein-binding fluorophores (PBFs) chosen by dye-screening approach. These PBFs are entrapped inside the nano-container and quenched by signal regulator. When encountered with $\cdot$OH, the antenna would trigger removal of regulator to release these entrapped PBFs, and thus successfully light up fluorescence. More importantly, intracellular proteins can immediately bind with these unencumbered PBFs and remarkably enhance their fluorescence. Due to cascade signal amplification, a ratio of maximum S/B > 800 for $\cdot$OH was achieved in cell lysates (Fig. 2). Moreover, OH-CA had been successfully used for tracking basal OH$\cdot$ levels and fluctuation in living cells (Fig. 3).

Reference:

Acknowledgements:
We are grateful for the financial support through the NSFC (21735001).

Keywords: Bioanalytical, Biosensors, Fluorescence, Imaging
Application Code: Bioanalytical
Methodology Code: Fluorescence/Luminescence
Protein vibrational spectroscopy could be an important tool in helping to unravel the mystery of its structure. However, this type of absorption spectroscopy has been hard to get due to the small optical cross section of proteins. In our work, self-induced-back-action (SIBA) trapping, a branch of optical trapping which doesn’t rely on the gradient force, can trap a single protein molecule without tags, labels, or tethering in the solution. It is an excellent label-free method and utilizes not only the near field enhancement of plasmonics but also the interaction strength between a trapped dipole and an optical resonator to be able to trap a single protein molecule. In this case, the optical resonator is either a double nanohole or an inverted bowtie nanoaperture antenna fabricated in 100 nm thick gold. Because this method does not rely on the gradient force, the input power does not exceed the damage threshold for trapping biomolecules. There are two effects on the transmission signal through the nanoaperture when a single biomolecule such as DNA or a protein is trapped. One, the transmission signal will increase as a function of the polarizability of the trapped molecule and two, there is an increase in the signal RMS (root mean square) due to both the intramolecular and the brownian motion of the trapped molecule. By using a lock-in amplifier, we can separate and isolate any change in the signal RMS due to the intramolecular motion and find the vibrational resonances of our trapped molecule. In this experiment, two lasers are used and if the beat frequency produced matches a vibrational resonance the signal RMS will increase. When the wavelength of one laser is tuned, vibrational modes of a single biomolecule can be measured over a large range and thus the structure of single molecule can be derived. Development of this work will provide a better understanding of the function of proteins.

Keywords: Molecular Spectroscopy, Nanotechnology, Raman Spectroscopy, Vibrational Spectroscopy
Application Code: Bioanalytical
Methodology Code: Molecular Spectroscopy
**Abstract Text**

Over the past decade, there has been a significant increase in the prevalence of neurodegenerative diseases. While the mechanisms into the cognitive and motor functions of these diseases have been studied extensively, the highly debilitating, early symptoms of anxiety and depression that accompany neurodegenerative diseases are not well understood. One challenge to understanding these symptoms is the lack of methods available to study the underlying neurotransmitters and ions in vivo. To better understand this problem, fast-scan cyclic voltammetry (FSCV) was used to measure serotonin and some ions in vivo in a variety of different neurodegeneration mouse models. We describe the development and application of FSCV in measuring ions such as Cu(II) in vivo. Additionally, we discuss data observing significant differences in the chemistry between normal and neurodegeneration models. Future studies aim to better understand the non-motor symptoms of neurodegenerative diseases.

**Keywords:** Metals, Neurochemistry, Sensors, Voltammetry

**Application Code:** Neurochemistry

**Methodology Code:** Electrochemistry
L-glutamic acid is one of the most prevalent excitatory neurotransmitter in the central nervous system with important roles in a variety of normal neuronal function, neurological and neurodegenerative diseases. While many methods have been employed to detect glutamate levels in the brain, amperometric measurements with a glutamate oxidase-based biosensor offers numerous advantages including high spatial resolution and real-time measurement. However, commercially available glutamate biosensors are expensive and larger in size. In this study, we report on the design and development of an amperometric microelectrode based glutamate oxidase biosensor for real-time monitoring of L-glutamate in vivo in the mammalian brain. In the first step, a polymer, poly-o-phenylenediamine (PPD) coating was electropolymerized onto 50 μm Pt wire to act as a permselective membrane to selectively block common interfering substances in physiological conditions. In the second step, a mixture of glutamate oxidase entrapped in a biocompatible chitosan matrix was casted onto the surface of a Pt microelectrode. In the final step, ascorbate oxidase was coated onto the microelectrode to eliminate almost completely of any interferences from high levels of extracellular ascorbic acid present in brain tissue. L-glutamate measurements were performed amperometrically at an applied potential of 0.6 V vs Ag/AgCl. The biosensor exhibited a very good linear dependence on the L-glutamate of 150 μM with a high sensitivity of 0.0966 nA/μM and excellent storage stability. The biosensor also showed a rapid steady state response to L-glutamate within 2 s, with a limit of detection 0.044 μM. Our results demonstrate that developed biosensor exhibited excellent analytical characteristics and was also successfully tested for stimulated glutamate measurements in the subthalamic nucleus of rat brain slices and in vivo and can also be employed for future neuroscience applications.
Characterizing the Impedance of Carbon-Fiber Ultramicroelectrodes and Microenvironments: Impact on Fast Scan Cyclic Voltammetry Measurements

Abstract Text

Background-subtracted fast-scan cyclic voltammetry employing carbon-fiber ultramicroelectrode (CFME) sensors has emerged as a powerful analytical technique for monitoring sub-second molecular fluctuations in tissue. However, the effects of impedance on these measurements, including shifts in impedance that occur over time and upon implantation in tissue, are not well characterized. Although noted, these effects have largely been ignored, leading to misrepresentation of data and hindering advances in quantification. Herein, we demonstrate that CFME impedance shifts upon implantation in tissue and is variable over time. The data show that uncompensated impedance systematically decreases the magnitude of the potential applied to the CFME surface, impacting sensitivity and voltammogram shape. This results in altered scan rates, adsorption properties, and surface conditioning. The use of potential offsets does not mitigate the issue. These findings are vital for improving data interpretation, calibration strategies, and sensor development for FSCV. As such, these findings will serve as a foundation for the development of new in situ calibration paradigms that will help to remove user bias from analysis and improve throughput.

Keywords: Bioanalytical, Biosensors, Calibration, Voltammetry
Application Code: Neurochemistry
Methodology Code: Electrochemistry
Developing Multichannel Electrochemical Electrodes and Instrumentation for Fast-scan Cyclic Voltammetry Measurements of Neurotransmitters

Modern measurement techniques such as fast-scan cyclic voltammetry (FSCV) and fast-scan controlled-adsorption voltammetry (FSCAV) use carbon fiber microelectrodes (CFME) to detect and measure dopamine. A major limitation of current electrodes is the localization to a single point in the brain based on proximity of dopamine to the carbon fiber electrode resulting in an incomplete understanding of the dopamine signaling processes. To overcome this single point limitation, current work has focused on the development of a carbon-fiber multi-channel array platform. A custom printed circuit board was fabricated to feature a 2x8 electrode placement with 300 µm spacing between fibers. Fibers were connected using either silver colloidal or carbon paint. Electrodes were insulated to have a defined electroactive area at the end of the fiber using a polypropylene mask and an electrodeposited insulator, ClearClad HSR©. The array can detect dopamine, and potentially other electroactive neurotransmitters at multiple points, which far exceeds the localization of a single carbon fiber electrode. Characterization of the array includes single channel analysis by slow scan voltammetry, FSCV, and FSCAV measurements of dopamine [i] in vitro[/i]. Future measurements and instrument development include thinner insulating coatings, hardware and software development for simultaneous multichannel recordings and [i] in vivo [/i] measurements of dopamine and other electroactive neurotransmitters such as serotonin.

Keywords: Electrochemistry, Instrumentation, Microelectrode, Neurochemistry

Application Code: Neurochemistry
Methodology Code: Electrochemistry
The most common method for bacterial infection identification uses culture plates, a process that takes 1 to 5 days before clinicians can confirm a bacterial infection and provide a targeted antibiotic to the patient. This delay in acquiring results can hinder patient care and contribute to the growing impact of antibiotic resistant bacteria. Therefore, rapid detection of bacterial infections at the point-of-care can play a key role in improving patient care and potentially preventing bacterial antibiotic resistance.

We will present a protocol for using low cost forward osmosis coupled with electrochemical sensing for rapid detection of low levels of bacterial quorum sensing molecules in bodily fluids. Disposable carbon-based electrochemical sensors were used to measure the concentration of pyocyanin, a redox-active molecule that is secreted by Pseudomonas aeruginosa. First, bodily fluids were spiked with known concentrations of pyocyanin and scanned using square wave voltammetry. The sample was concentrated for less than an hour using a low-cost forward osmosis setup and rescanned to determine the final pyocyanin concentration. A 4-fold increase in signal was measured within 40 minutes using this technique with an up to 150-fold increase in signal with repetitive runs. We expect that this approach will pave the way for faster and earlier detection of bacterial infections while maintaining a simple and low cost sensing system.

Keywords: Biological Samples, Biosensors, Electrochemistry, Voltammetry
Application Code: Clinical/Toxicology
Methodology Code: Sensors
Signal Summing for Improved Mass Spectrometric Detection of High Abundance Isotopologues

This research sought to explore several applications of signal summing and its potential benefits in the context of an analytical method for the quantification of urinary metabolites of volatile organic compounds (VOCs). While still relatively unexplored in the field of mass spectrometry, signal summing is an inexpensive, adaptable, and easy to implement methodology for improved analytical sensitivity. This methodology was applied for the quantitation of two VOC metabolites with an existing UPLC-ESI-MS/MS method. N-Acetyl-S-(2-carboxyethyl)-L-cysteine (CEMA), a urinary metabolite of acrolein, was quantified using signal summing of two abundant MS/MS transitions with the same of the precursor ion (i.e., 234 162 and 234 105). Moreover, quantitation was performed by the addition of multiple scans of the same ion transition (i.e., 234 162, n 2). By monitoring five scans of the same ion transition, the S/N ratio was improved by 31% in the urine matrix, leading to a corresponding decrease in the limit of detection. The effects of signal summing were extended to multiple high abundance ion transitions of isotopologues of N-acetyl-S-(1,2-dichlorovinyl)-L-cysteine (1DCV), a metabolite of trichloroethylene exposure. Due to the relatively high abundance of stable isotopes of chlorine in 1DCV, only 49.95% of 1DCV is quantitated when a single monoisotopic ion transition (i.e., 256 127) is monitored. By incorporating signal summing of another high abundance ion transition (i.e., 258 129), the S/N ratio was improved by 13% in the urine matrix, leading to a corresponding decrease in the limit of detection. Mr. McCarthy, Mr. Movassaghi and Mr. Geldner were funded by the Research Participation Program at the Centers for Disease Control and Prevention, an interagency agreement with the US Department of Energy administered by the Oak Ridge Institute for Science and Education.

Keywords: Clinical/Toxicology, Mass Spectrometry, Volatile Organic Compounds, Metabolomics
Application Code: Clinical/Toxicology
Methodology Code: Liquid Chromatography/Mass Spectrometry
Undergraduate analytical chemistry courses emphasize fundamental stoichiometric and physico-chemical analytical techniques with linear calibrations. Higher-level data analysis techniques are not often included in the college junior-level curriculum, but widely available software enables more complex analysis to be accessible. Activities to train students in data linearization (fitting nonlinear data to a linearized model), multi-component spectral curve-fitting, and classical least squares (utilizing matrix algebra) were incorporated within a large-enrollment undergraduate analytical chemistry lecture setting. When analyzing multiple compounds in mixtures without separation pre-treatment, both non-linear curve fitting and classical least squares are valuable techniques for students to understand and execute using commercially available software. By implementing hands-on activities, multi-media screencasts, and in-class data collection and analysis, students were trained to employ the advanced analysis methods. The efficacy of the in-class practical activities were assessed with pre- and post-test instruments that quantified gains in students’ knowledge. Inclusion of such activities will empower students with an expanded repertoire of these important analytical analysis methods and their applications with a real world, portable, hands-on approach that can be completed in a lecture setting with non-hazardous samples.
Experimental work plays a crucial role in higher chemical education and goes hand in hand with proper theoretical training. The reason for its essential importance lay in the need of skilled experts for research laboratories in academia and industry. Within the past decade the requirements on specialists in this field changed significantly since laboratory infrastructures were evolving constantly. The industrial 'Internet of Things' is about to hit the biotechnological and chemical field just as hard as recently the manufacturing industry. Research institutions are implementing laboratory information management systems (LIMS) in order to efficiently handle big data and data analysis. Higher education is challenged to adopt to the novel requirements to properly prepare students for their professional life.

In this work we present a novel lab course, where students ought to learn to deal with innovative technologies embedded into a chemical engineering experiment. Process parameters of an STR like mixing time, impeller Reynolds Number and Newton Number need to be determined. Therefore, participants design and additively manufacture impellers using Fused Deposition Modeling (FDM) technology yielding high quality results just-in-time. The impellers are afterwards characterized in a custom experimental setup. The experiment is assisted by the usage of smartglasses and a tablet for the display of the experiment procedure and for workflow documentation and data management. The smartglasses are directly attached to the experimenter's safety goggles and experiment parameters (e.g. SOP, safety information) are displayed directly in the operator's field of view. The voice recognition capabilities of the involved smart mobile devices ensure an easy and convenient control during the lab work. Relevant data are automatically stored in a database. The combination of traditional fields of chemical engineering and innovative technologies can be an asset to practical education.

Keywords: Biotechnology, Education, Laboratory Automation
Application Code: Other
Methodology Code: Education/Teaching
Mass spectrometers are at the forefront of analysis in academia and industry. From identifying proteins to optimizing industrial processes, mass spectrometers are key to thorough analysis of common and complex processes. Students often have difficulty bridging the connection between the classroom and “real-world” applications. The qualitative and quantitative data provided by the mass spectrometer can lead to a deeper understanding of the processes and chemicals commonly used by students in laboratory analysis. When paired with a real world understanding, students can develop a well-rounded understanding of how these analyzers work.

The presentation is geared towards students and educators alike featuring mass spectrometry based laboratory modules applicable to various studies in the realm of chemistry. Discussion focuses on how instrument design determines the outcome and quality of the educational experience. Example data that addresses a number of qualitative and quantitative applications of gas analysis are discussed within the larger context of how they prepare students for success in a variety of scientific fields.

Keywords: Education, Mass Spectrometry, Quadrupole MS, Teaching/Education

Application Code: Other

Methodology Code: Education/Teaching
Biomimicking Colloidosome-Based Dual-Enzyme System for Efficient Oxidation of N-Heteroaromatic Compounds

Colloidosomes are microcompartmental structures with semipermeable membranes. As a type of synthetic cellularity, colloidosomes can be used to develop biomimicking enzyme system for efficient catalysis. Herein, a double-phase catalysis system based on Glucose oxidase (GOx), and Lipase B from Candida antarctica (CalB) was designed and developed for efficient oxidation of N-heteroaromatic compounds. Amphiphilic silica nanoparticles functionalized with octadecane were used to fabricate amphiphilic inorganic microcapsules from water-in-oil Pickering emulsions, as the building blocks to separate the double-phase catalysis system. Aqueous solution of GOx was doped inside the microcapsules and CalB was adsorbed on the outer surfaces of the microcapsules. To investigate the fabrication of the dual-enzyme system, dye-doped silica nanoparticles were used as the building blocks. Fluorescent microscopy were employed to formation of the colloidosome, Glucose was catalyzed by GOx to produce H2O2 in aqueous phase, then H2O2 was caught by CalB to develop the oxidation of ethyl acetate after diffusing out the microcapsules to produce peracid. The catalysis was studied using UV-Vis spectroscopy and gas chromatography. According to the results of thin-layer chromatography and gas chromatography, pyridine, as a model molecule of N-heteroaromatic compounds, was able to be oxidized by peracid in situ.

Keywords: Fluorescence, Gas Chromatography
Application Code: General Interest
Methodology Code: Fluorescence/Luminescence
The strong light-matter interaction that occurs between a single dye molecule and a plasmonic nanoantenna has attracted tremendous attention due to the resulting strong fluorescence enhancement, which can improve the resolution of single molecule imaging. These plasmonic nanoantennas are metal nanoparticles that transfer free-space, far-field radiation to localized electromagnetic energy. Besides the fluorescence enhancement, we have discovered that the far-field emission pattern of a dye molecule is also largely modified when coupled to plasmonic nanoantennas. In particular, using single-molecule microscopy, we have measured a large difference in the emission polarization from single Cy5.5 molecules upon coupling to a plasmonic gold nanorod. To our knowledge, this is the first study that shows deliberate tuning of the emission polarization of single molecules via nanoantenna coupling. This study will improve high-sensitivity single-molecule fluorescence imaging by increasing the tunability and control of plasmon-enhanced fluorescence, and it will further provide an in situ probe for measuring—and controlling—light-matter interactions on the nanometer scale.

**Keywords:** Biosensors, Fluorescence, Microscopy, Molecular Spectroscopy

**Application Code:** Nanotechnology

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

In the world of international ISO standards, many are now welcoming the publication of the revision to ISO/IEC 17025 in 2017. It has been 12 years since the prior version was introduced. Many of the referenced documents in the 2005 version standard have by now been obsoleted, and a change was desperately awaited. Now that it is published and in place, tens of thousands of labs both accredited and applicants are scrambling to learn what is new and different.

There are a number of considerations for laboratories to take in their transition to or preparation for the 2017 standard, such as obtaining official copies of the published standard, training of key staff and getting documents and forms in place within the internal quality management system.

For laboratories that already have documentation based on the older version, there is a great benefit to obtain or develop a crosswalk document, identifying all the changes between the two versions of the standard.

Key to the process of preparation is learning transition plan from the laboratories’ accreditation body (AB), associated timing and practices required. Although the international deadline is the same for all, elements of transition could differ from AB to AB.

One of the biggest challenges to accreditation bodies is to define the implementation of the management system auditing for their customers who maintain ISO 9001 in addition to ISO/IEC 17025. It is obvious that not all the management system requirements of ISO/IEC 17025 are found in ISO 9001. Each AB must decide which additional ones need to be verified for their 9001/17025 customers. Accompanying this is to decide how much assessment time saving there may be to customers in this situation.

This presentation will outline key changes in content, format and style in the new revision. It will focus on impact of changes on laboratories as well as their customers.

Keywords: Lab Management, Quality, Quality Control, Standards
Application Code: Laboratory Management
Methodology Code: Education/Teaching
Single-particle aerosol MS (ATOFMS) based on laser desorption/ionization (LDI) is a versatile method for characterization of airborne particles. Currently available ATOFMS-technologies detect mainly inorganic species. Recently also ATOFMS-approaches for an on-line single particle detection of polycyclic aromatic hydrocarbons (PAH) were developed (Bente et al., Anal.Chem. 2008, 8991ff). The particles are desorbed by an IR-laser (LD) and subsequently the PAH are ionized by resonance-enhanced multiphoton ionization (REMPI with UV-laser pulse) for MS detection. A drawback here is, however, that the LDI-information on the inorganic particle-composition is lost. Here we developed a new multi-step laser ionization ATOFMS-concept for detection of organic species (LD-REMPI) and element signatures (LDI) from the same individual aerosol particle. The approach is based on the sequential application of laser pulses and extraction field polarity-inversion of the dual-TOF mass analyzer between the LD/REMPI- and LDI-laser ionization processes. The particle in the ion source is desorbed by an IR-laser pulse and the PAH subsequently are ionized by the UV-laser pulse (REMPI). However, after some 100 ns, when the PAH-ions have left the ion source, the field in the ion source is reversed and the very same particle core, which is still on its fly-through path in the ion source, is hit by a third, intense laser pulse for LDI. Due to the inverted field the LDI-cations now are accelerated in the second TOF analyzer. By this procedure in addition to the particle size, the REMPI-spectrum of the absorbed PAH-molecules as well as the LDI-spectrum (showing e.g. Fe+ or Al+) from the very same individual particles can be detected. First measurements of wood combustion- and diesel-emission particles and ambient particles are shown. On-line multi-step ATOFMS represents a promising technology for source apportionment and internal and external mixing state-analysis of inorganics and organics.

Keywords: Aerosols/Particulates, Elemental Analysis, Mass Spectrometry, PAH
Application Code: Environmental
Methodology Code: Mass Spectrometry
Labeling of antibodies with fluorophores is a common precursor to immunostaining, immunoblotting, and other detection techniques. The degree of derivatization must be precisely controlled to maintain optimal function of the labelled antibody; this is accomplished through a series of experiments performed with varied mole ratios of dye to protein (e.g. 10 – 2-fold excess), consuming a substantial amount of reagent. A simple, rapid analysis of protein labeling is needed to conserve time and reagents, and optimized reagent ratios should be directly scalable for lab use. SlipChips offer a unique solution to these issues by providing a hand-operated platform that requires no specialized equipment or training to operate. To perform and analyze multiple labeling reactions in parallel, we designed a SlipChip comprised of two glass layers. The glass was wet etched to multiple depths to provide precise volumetric control during use. Antibody and dye were loaded onto the device by pipet, using < 5 µL of protein at concentrations as low as 1 mg/mL. The first slip of the device performed a precise dilution of dye. In preliminary work, the device performed a 1 to 5-fold dilution of a model dye (0.1 M FeSCN), yielding a linear concentration range with respect to absorbance \( R^2 = 0.994 \). A second slip mixed the dye with protein sample and then the device was slipped again to remove un-reacted dye from the sample. Wells filled with crosslinked polyacrylamide gel were used as a molecular weight filter, excluding protein sample and extracting the dye. Preliminary results showed that 20% polyacrylamide gel was capable of removing a substantial amount of dye while excluding antibody sample. In summary, we have separately shown accurate dilution of dye on chip, mixing of dye with sample, and removal of dye from sample. In future work these processes will be integrated to provide rapid, small-scale optimization of protein derivatization, and be compatible with UV-VIS detection.

Keywords: Derivatization, Fluorescence, Lab-on-a-Chip/Microfluidics
Application Code: General Interest
Methodology Code: Microfluidics/Lab-on-a-Chip
Many children are breathing in secondhand smoke (SHS) in their own homes and public places. In addition, children are more susceptible to the negative effects of secondhand smoke as they normally breathe faster than adults do. The methods commonly used for assessing the exposure to SHS are based on detecting specific biomarkers of tobacco smoke. These analytical procedures are often labor-intensive and time-consuming, leading to lengthy and costly analysis, thus are not suitable for home-based test. To address this challenge, we propose to develop a paper-based analytical device (LPAD) for detecting SHS exposure. Cotinine, a predominant metabolite of nicotine, is chosen as the detection target in our research. Our device contains two major components, one of which is an enrichment component and the other is an analytical component. Since there is always a lower cotinine concentration in nonsmokers’ blood and urine samples than smokers’, it will be very helpful to decrease the limit of detection (LOD) of a cotinine detection device using a concentrating component. We concentrate the sample by evaporating it on a super hydrophobic material and then detect the concentrated cotinine on paper using an immunoassay. The detailed designs and results will be presented in the poster.

Keywords: Analysis, Detection
Application Code: Bioanalytical
Methodology Code: Microfluidics/Lab-on-a-Chip
Proper conditioning of the inner capillary surface in capillary electrophoresis (CE) is of utmost importance for the run-to-run reproducibility of the migration times and peak areas of the analytes. Typically this is accomplished by the capillary rinse.

To assess the quality of the rinse we propose to measure a streaming potential (SP), a phenomenon which arises between the ends of a fused silica capillary when electrolyte is pressed through it. A corresponding hardware and software were implemented in the commercial Capel-205 CE system (Lumex Instruments, Canada). First, a new capillary is thoroughly rinsed and afterwards a so called exemplary SP value is saved in a capillary certificate. Later during the sequence of analyses, SP is automatically measured every time after rinsing and the software compares it with the exemplary value, thus deciding whether or not to continue the rinsing.

This approach can be used not only for the fundamental research, but also to provide long sequences of CE analyses overnight in a fully automated way. Even if a capillary has become contaminated during one of runs and a standard rinse did not clean it properly, the system can automatically start another type of rinsing based on the streaming potential values.

Another perspective application field of streaming potential control is the assessment of the integrity and stability of any kinds of the capillary coatings.

Keywords: Automation, Capillary Electrophoresis, Characterization
Application Code: Quality/QA/QC
Methodology Code: Capillary Electrophoresis
Diagnostic applications of polymerase chain reaction (PCR) have been developed over the last decades based on the achievements in analytical microchip systems. Current PCR analysis techniques for multiple pathogen detection are time-consuming, require tedious reaction setup and prone to user errors. Recently developed microchip-based real-time PCR systems offer accurate, robust and cost-effective detection of different pathogens related to diseases of plants, animals, and humans.

We developed a microchip-based PCR technology that allows to decrease analysis time down to 15 minutes (45 cycles), while microchips with lyophilized PCR reagents decrease reagent consumption 10-30 times and decrease user errors.

Microchips with lyophilized PCR reagents allow analyzing nucleic acids without labor consuming pipetting steps. The shelf-life of microchips with PCR reagents is up to 6 months under ambient temperature conditions making the transportation and storage of the microchips easier and leading to more reliable results.

To meet requirements of microchip PCR device, simple and fast nucleic acid isolation from samples is also essential as an upstream method. Several nucleic acid extraction methods using commercially available kits were optimized for microchip-based PCR and their applicability for automated sample preparation was shown.

It has been experimentally demonstrated that microchips are efficient for determination of several pathogens in several samples (from 2 up to 14 on a single chip). Identification of pathogens with a microchip-based real-time PCR analyzer AriaDNA provides high sensitivity and specificity of detection.

The results of direct determination of pathogens in real samples show a good correlation with other analytical techniques such as ELISA and test-tube based PCR, and will be discussed in details in presentation.
Microbiological culture remains the most sensitive method for detecting viable and infectious bacteria, but often requires 24 hours to identify growth. Lab-on-a-chip applications have been developed to isolate bacteria in picoliter-sized reaction vessels, or droplets, resulting in digitized signals with improved time-to-detection and quantification. Although a great development, these approaches require expensive and specialized equipment, trained personnel, and limited volumes that can be orders of magnitude less than needed for clinically relevant limits of detection.

To overcome these limitations, we developed a simple, low-cost method for droplet generation and in-droplet bacterial culture. Our method is based on bulk emulsion formation, uses off-the-shelf components, and is compatible with a wide range input sample types. Also, this system can function with a range of volumes and uses the entire sample, therefore increasing total addressable volume. This method can use simple hand-mixing to prepare droplets, removing the constraints of laboratory equipment, electricity, and trained personnel. However, it is not limited to hand-made droplets, and can be used with a laboratory vortexer.

Our method accurately quantified input bacteria counts and resulted in improved time-to-detection over traditional bulk-based culture. The droplets prepared with this system also remained stable for up to 144 hours, indicating that these methods could be compatible with growth of mycobacterium. We also characterized the range of polydisperse droplet sizes created and present statistical methods to quantify the number of bacteria in these samples. Importantly, this statistical approach enables quantification of bacteria in a sample without having to measure the actual droplet distribution. The combination of rapid droplet preparation and statistical methods leads the way for implementation in a variety of settings for more rapid detection of antimicrobial resistance.

Keywords: Bioanalytical, Biosensors, Lab-on-a-Chip/Microfluidics
Application Code: Bioanalytical
Methodology Code: Microfluidics/Lab-on-a-Chip
Single walled carbon nanotubes (SWCNTs) are emerging as electronic materials in flexible electronics, sensors and other applications. As synthesized SWCNTs are typically impure and consist of many electronic types. Samples are subsequently processed and sorted, for example by polymer wrapping, to obtain highly pure material with the desired electronic properties. To validate and improve these highly purified materials, characterization tools are required. Raman spectroscopy is one of the most established characterization tools for SWCNTs and other nanocarbons, and it is has great potential for assessing these highly pure materials. It is important now to determine the extent to which purified samples (e.g. semiconducting SWCNTs) remain contaminated with undesirable species (e.g. metallic SWCNTs). Raman excitation mapping, whereby the Raman spectra are obtained for many excitation wavelengths, provides a sensitive way to identify species and electronic type, through the Kataura plot. Here, tunable filters are employed as a practical method making Raman excitation maps of highly purified material and identifying trace contaminants. State-of-the-art SWCNT materials are evaluated in this way.

Keywords: Nanotechnology, Raman Spectroscopy
Application Code: Nanotechnology
Methodology Code: Vibrational Spectroscopy
Probing Molecular Transport in Nanoporous Silica Particles by Single-Molecule Spectroscopy

Abstract Text

Single-molecule spectroscopy is used to study the molecular transport in nanopores, which provides a better understanding of the molecular processes that control the functional applications of the nanoporous materials utilized in chemical separations, drug delivery, biosensing and environmental remediation. Single molecule photon bursts data are collected from 100 locations in the nanopores of 19 particles to build a statistical population for analyzing the adsorption, diffusion, and local concentration of rhodamine 6G molecules in nanopores. A home-built confocal instrument is used for data collection. Surface heterogeneity within the particle population is discovered by analyzing the molecular transport information in various particles. The results show that there are not significantly more strong adsorption events found in underivatized silica nanopores compared to C18-derivatized silica nanopores, which indicates the peak tailing in reversed-phase liquid chromatography is not contributed by the strong adsorption on the underivatized sites, from the single-molecule experiment perspective. Additionally, the micro-second temporal resolution of the proposed single-molecule measurements allows the revealing of shorter adsorption events with desorption time under 5ms, which provides a higher level of details in molecular adsorption in nanopores compared to previous single-molecule studies.

This research is supported by Graduate College Summer Fellowship offered by The University of Iowa.
Atomically precise gold nanoclusters consisting of up to a few hundred gold atoms have exciting intermediate electronic structures between molecular and bulk metallic gold. Nanoclusters with solved crystal structures are vital to understanding the fundamental physical principles within this material class. Here, we systemically exchange halides on one of the smallest atomically precise gold nanoclusters, Au_{11}(PPh_{3})_{8}X_{2}^{+} (X = Cl, Br, I). The resulting structures are validated by X-ray crystallography and electrospray ionization mass spectrometry. Both the absorption spectroscopy and computational results using density functional theory (CP2K) indicate that the particles are opto-electronically similar; the HOMO states of the Au_{11} clusters are mostly dominated by electron density from gold, especially near the core, but are also measurably influence by the presence of a halogen in the ligand shell. The impact of halogens in these clusters is then compared to mononuclear gold precursor analogues (PPh_{3}-Au-X), where electron densities of the HOMO states are usually populated near the halogens and the LUMO states have more contributions from the organic component. Because these nanoclusters have similar structures and absorption properties, photoluminescence (PL) experiments in Au_{11} clusters can provide information on the impact of halogen ligands on emission pathways in nanostructures. PL experiments on Au_{11} clusters show that as halides get heavier, the emission from the Au_{11} clusters blueshifts and increases in quantum yield with decreasing radiative lifetime. These spectroscopic changes can be attributed to the heavy atom effect observed in organic molecules in which larger atoms promote phosphorescence, which is thought to be a component of the gold nanocluster emission mechanism.

Keywords: Luminescence, Nanotechnology, Near Infrared, UV-VIS Absorbance/Luminescence
Application Code: Nanotechnology
Methodology Code: Fluorescence/Luminescence
We demonstrate an integrated dielectrophoretic (DEP) line-Raman platform for multiplexed isolation and surface-enhanced Raman (SERS) imaging of 50 nm liposomes within seconds. There is strong evidence for nanometer-scale extracellular vesicles, such as exosomes and liposomes, to be important indicators of cancerous activity and pathological signaling within the body. Such vesicles and their internal cargo carry valuable information regarding the state of their host cell. While current isolation and detection schemes of nanovesicles require extended centrifugation and chemically specific binding assays, our method is label-free in both DEP trapping and SERS imaging. Utilizing a 40-μm-long and 10-nm-wide gold nanogap electrode, nanoparticles are trapped within seconds to the gold surface at a bias voltage of 600 mV, which is an order of magnitude smaller than typical voltage required for microelectrodes. A laser-line illumination source generated using anamorphic optics is aligned to the gap to provide Raman excitation and is imaged along the entire 40-μm region with one second exposure—100 times faster than a similar point-scan Raman system. This provides delay-free temporal and spatial information regarding multiplexed trapping events and real-time SERS states. Surface-enhancement of the Raman spectra was achieved by forced interactions of the liposome within the high-field point junctions of 70 nm gold nanoparticles which are spiked into solution and simultaneously trapped. Our design requires no chemical functionalization, is faster at isolation and SERS imaging than current techniques, and has promising applications for rapid and sensitive detection of nanovesicles from biological samples. This research was supported by the National Science Foundation (NSF ECCS No. 1610333) and the Minnesota Partnership for Biotechnology and Medical Genomics. C.T.E. also acknowledges support from the NSF Graduate Research Fellowship Program.

From 2019, four phthalate esters (DIBP, DBP, BBP, DEHP) will be added as restricted substances in the RoHS directive. After solvent extraction of a polymer sample, GC/MS is widely used for quantitative determination of phthalates esters. However, the conventional solvent extraction GC/MS method is not suitable for RoHS testing since the number of samples for phthalate analysis will increase dramatically because of the need to check over several hundreds of parts that are included in one electrical/electronic product.

To solve the problem, Maruyama, et al1) reported the effective phthalate analysis system using pyrolyzer/thermal desorption-GC/MS (Py/TD-GC/MS) which does not require organic solvent for the sample preparation. This analysis method is described in IEC62321-8 published on March 2017 as the international standard method of phthalate esters screening. In addition, we developed novel reference material consisting of seven phthalate esters (DIBP, DBP, BBP, DEHP, DNOP, DNP, DIDP, conc: blank, 100, 1000 mg/kg each) for Py/TD-GC/MS, which can be prepared easily using a micro puncher. This material can be used for QC and calibration curve creation to increase the productivity of the RoHS testing process.

In this study, the quantitative accuracy of phthalate ester analysis using Py/TD-GC/MS and the reference material were evaluated to confirm the applicability of phthalate screening for RoHS testing. The results demonstrated that Py/TD-GC/MS with the novel reference material enables highly sensitive and productive screening of phthalate esters, which is suitable for RoHS testing analysis.


Keywords: Gas Chromatography/Mass Spectrometry, Polymers & Plastics, Reference Material, Thermal Desorption
Application Code: Polymers and Plastics
Methodology Code: Gas Chromatography/Mass Spectrometry
The acryl resin was prepared with metal-organic acid copolymer for synthesis of acryl resin-metal-organic acid coordination complex. Metal-carboxyl type (RCOO)-X-(OOCR'), where R represents a hydrolytically stable backbone carboxyl group of acryl resin, and R' is a soluble functional organic acid and X is a metal linking resin and organic acid, are interesting precursor for the preparation of novel resin of antifouling paint composed of both inorganic and organic entities. Three main products are separated on the Prep-LC system and these complexes were qualitative analyzed by FT-IR spectroscopy. To determine the structure of each product, we used nuclear magnetic resonance spectroscopic techniques such as diffusion ordered spectroscopy and two dimensional nuclear Overhauser effect spectroscopy. We also try to measure the average molecular weight using laser light scattering analysis. In this study, we successfully identified these main products constituted of acryl resin-metal-acryl resin, acryl resin-metal-organic acid, and organic acid free form, and it will be aid for analysis of hybrid polymers having both inorganic and organic moieties.
Silsesquioxanes (SSQs) are versatile materials that can form cross-linked networks composed of silicate linkages and organic substituents. SSQs are utilized more frequently in nanoscale patterning applications because they offer many advantages in terms of the mechanical and physical properties important for nanoscale patterning media. Most of the time, the patterning process requires the SSQs to be used in solution. The precipitation of SSQs can lead to major changes in the chemical and physical (hence performance) properties. It is a great challenge to characterize the SSQs in solution without changing the sample integrity since SSQs are very sensitive to water and temperature. NMR is a versatile and non-destructive technique that can characterize the materials without complication from sample preparation. In this study, Diffusion-filtered 1H NMR and T2-filtered 29Si NMR were used to characterize the SSQ polymer of Methyltrimethoxysilane (MTMS) and Tetraethyl orthosilicate (TEOS) in Propylene Glycol Ethyl Ether (PGEE) solution. Detailed structure characterization of polymer in PGEE by NMR had been hindered by the undesirable signals from low Mw species masking the polymer signals. Diffusion filtered 1H NMR, employing pulsed field gradient (PFG) NMR methods, is a powerful tool to delineate signals of various molecules based on their self diffusion coefficients. The diffusion-filtered PFG 1H NMR parameters are carefully optimized to remove or significantly attenuate the undesirable signals of low molecular weight species, while to preserve the polymer signals as much as possible. T2-filtered 29Si NMR was utilized to remove the broad background signals from NMR probes and tubes. In this study, the amounts of silanol, Si-OCH3, and Si-OCH2CH3 of the SSQ polymer were obtained. Using T2-filtered 29Si NMR, the amounts of the different T and Q species were obtained. A formula was also developed to calculate the condensation level of SSQ polymer in solution.

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

**Application Code:** Polymers and Plastics

**Methodology Code:** Magnetic Resonance
Depending on the globalization of supply-chain of (source-) materials, a risk of “silent change” has become apparent recently. To avoid the risk, rapid screening technique for various kinds of materials is required. The DIP/MS (direct inlet probe/mass spectrometry) is one of the solutions for the rapid analysis as screening technique. However DIP requires low vacuum just before the measurement, so that some chemical compounds disappear due to the low boiling point. Therefore the sample productive method has been developed to guard against the vaporizing of the low boiling point compounds during evacuation of the DIP chamber, and it has been applied for analysis of phthalic acid esters. Ion attachment ionization mass spectrometry that is one of fragmentless ionization mass spectrometry offers rapid analysis as screening. In the mass spectrum, one peak indicates one chemical species because no fragmentation exists during the ionization, so that the separation is realized in the mass spectrum. Phthalic acid esters mixture (1~10 [micro]L) were set into specimen cell (SUS, 5 mm[Phi]×2.5 mmH) and vaporized its solvent at room temperature. After that, poly(vinyl butyral) solution (10 [micro]L) coated the phthalic acid esters. After vaporizing the solvent at R.T. leaded to PVB-covered specimen. The coating could guard against the vaporizing of the low boiling point compounds. 6 kinds of phthalic acid esters, benzoic acid and methyl benzoate were measured for demonstration of the sample productive method. With the PVB coating, all samples offered no gasification before heating-start. It means the PVB coating worked well to guard against the vaporizing of the analytes. The amounts of phthalic acid esters corresponded to from 0.01 to some wt%. It is enough sensitivity for, e.g., RoHS Directive. The required time of this measurement is within 15 min that is total of 3-5 min for coating, 1.5 min for setting specimen to apparatus, 8.5 min for measurement.

**Keywords:** Mass Spectrometry, Materials Characterization, Polymers & Plastics, Sample Preparation

**Application Code:** Polymers and Plastics

**Methodology Code:** Mass Spectrometry
In our design, we design two DNA probes, P1 and P2 which P1 and P2 can complementary with a target DNA. Then P1 was modified with catalysis on the 3 primer end, and P2 was conjugated with active small molecules on 5 primer end. On the original state, the catalysis was capped with a hydrophobic molecule to quench its catalytic ability. However, when the target DNA shows up, P1 and P2 will complementary with target DNA that will proximity the capped catalysis with active molecules. Because of the high binding ability between cap molecules with active molecules, the active molecules will remove cap molecules to recovery the catalytic ability of catalysis. At this situation, the catalysis can catalyze dye and prodrug for cancer imaging and cancer therapy. By this method, we can amplify the single to detect the traceless DNA species in vitro since one catalysis molecule can catalyze a lot of reactions. On the hand, it also can be used for target cancer therapy which only active prodrugs in the cancer cells.

Keywords: Analysis, Biosensors, Detection, Imaging
Application Code: Bioanalytical
Methodology Code: Sensors
Exosomes constitute an emerging biomarker for cancer diagnosis. They carry multiple proteins that reflect the origins of parent cells. Assessing exosome surface proteins provides a powerful means of identifying a combination of biomarkers for cancer diagnosis. Here we report a sensor platform that profiles exosome surface proteins in minutes by the naked eye. The sensor consists of a gold nanoparticle (AuNP) complexed with a panel of aptamers. The complexation of aptamers with AuNPs protects the nanoparticles from aggregating in a high-salt solution. In the presence of exosomes, the non-specific and weaker binding between aptamers and the AuNP is broken, and the specific and stronger binding between exosome surface protein and the aptamer displaces aptamers from the AuNP surface and results in AuNP aggregation. This aggregation results in a color change and generates patterns for the identification of multiple proteins on the exosome surface. This, then, is a universal strategy to identify constituent proteins of exosomes secreted by different cell types, thus providing an effective exosome-based platform for sensing and disease diagnosis.

Keywords: Bioanalytical, Biosensors, Identification, Nanotechnology
Application Code: Bioanalytical
Methodology Code: Sensors
Detection of genes can provide very useful information for clinical diagnosis of many serious diseases. Currently, the most widely used gene detection method is the real-time quantitative polymerase chain reaction, which has high sensitivity. However, it needs expensive instruments, laboratory reagents, professional operators and is a time consuming process. Therefore, it is very important to develop highly sensitive, low cost and rapid gene detection methods. In this work, we employ fluorescent carbon quantum dots coupled with loop-mediated isothermal amplification (LAMP) to detect multiple-resistant [i]Staphylococcus[/i] [i]aureus[/i] (MRSA). First, we synthesized fluorescent spermidine-carbon quantum dots (CQDs[sub]spd[/sub]) by a simple one-step synthesis method. Then the target gene ([i]mecA[/i] gene) fragment in clinical sample was amplified by LAMP, followed by treatment with positively charged CQDs[sub]spd[/sub]. The DNA, upon binding with the CQD[sub]spd[/sub], effectively quenched the fluorescence, which is used as the probe response mechanism to determine whether the sample is infected with MRSA. The CQDs[sub]spd[/sub] probe can be used for both qualitative and quantitative determination of bacteria. The limit of detection (LOD) of the probe is 10[sup]2[/sup] CFU/mL. Compared with the traditional detection methods, the CQDs[sub]spd[/sub] nanosensor is simple, fast, low cost, highly sensitive and specific for clinical diagnosis. This method may be applied to detect other pathogens, gene methylation analysis and cancer screening.
We report here the use of a polymerization reaction as a signal amplification method to visualize gender-specific genomic DNA markers directly extracted from human blood without polymerase chain reaction (PCR). Clear distinction between X and Y chromosomes was made both qualitatively by naked eyes and quantitatively by ellipsometry. Assay optimization was conducted, including the selection of the proper blocking reagents, annealing temperature, and annealing time. For performance comparison, traditional PCR was carried out in parallel, followed by gel electrophoresis for amplicon identification. Among 26 blood samples examined, 25 were blind-tested correctly and one was false negative, which was comparable to, if not better than, the PCR outcomes. The results confirm the potential of the described sensing technique in eliminating PCR for complex genome DNA analysis, which advances one step closer to the development of a portable DNA sensing device for point-of-need applications.

**Keywords:** Bioanalytical, Biosensors, Polymers & Plastics, Sensors

**Application Code:** Bioanalytical

**Methodology Code:** Sensors
Resolving spectrally adjacent peaks is important in techniques such as tracking small shifts in Raman or fluorescence spectra, quantifying pharmaceutical polymorph ratios, or molecular orientation studies. Thus, spectral resolution is a vital consideration when designing most spectroscopic systems. However, most of the parameters that influence spectral resolution are fixed for a given system (spectrometer length, grating groove density, excitation source, CCD pixel size, etc). This is non-problematic if the spectrometer is dedicated for a single purpose; however, these specifications cannot be optimized for different applications with wider range resolution requirements.

Data processing techniques including peak fitting, PLS or PCA are typically used to achieve sub-optical resolution information. These techniques can be plagued by spectral artifacts introduced by post-processing as well as the subjective implementation of statistical parameters. TrueRes[\textsuperscript{circumflex O}], from Andor Technology, uses an innovative optical means to manipulate the input f-number of the spectrograph and greatly improve and expand the range of spectral resolutions accessible on a single spectrometer parameter setup. This increases the versatility of the spectrometer and allows one to fine tune the spectral resolution without sacrificing spectral bandpass.

Data presented here shows a true spectral resolution enhancement of >30% without mathematical spectral alteration, data-processing or spectrometer component changes. The resolution enhancement is characterized using Raman emission of a characteristic analyte with spectrally-adjacent peaks. The total throughput change and improvement in hyperspectral imaging quality is also characterized. We also show that TruRes[\textsuperscript{circumflex O}] allows imaging of several individual fibers on both sides of the CCD detector. The enhanced resolution enables the spectrograph to produce a higher hyperspectral track density by sacrificing some throughput.
Microfluidic carbon paste electrodes are easily fabricated and inexpensive electrochemical sensors that consume very small volumes (nL) of samples and reagents, yet produce high resolution and sensitivity within a short analysis time. The traditional carbon paste electrode combines carbon (graphite) with a binder (mineral oil). However, recent studies suggest that modifying electrodes with different carbon materials and binder, along with heat or plasma treatment could vastly improve the electrochemical behavior of the electrode. We investigated different carbon paste mixtures fabricated with different proportions of graphite or graphene and different proportions of ionic liquid (IL) or mineral oil binder. Cyclic voltammetry of two redox species, ferricyanide and to tris(2,2'-bipyridyl)ruthenium (II), was used to characterize the electrochemical behavior of the electrodes. Parameters such as the peak separation, peak current and peak current ratio were calculated. The results indicated that mixing a 50:50 ratio of mineral oil and ionic liquid enhance the performance of graphite carbon paste electrodes. Graphene and mineral oil produced the top-performing device (working, counter and reference electrodes all the same material) overall; while graphite and ionic liquid produced the best working electrode (silver-silver chloride reference). Future work will compare these electrodes for use in electrochemiluminescent devices.

Keywords: Electrochemistry, Electrodes, Lab-on-a-Chip/Microfluidics, Voltammetry
Application Code: General Interest
Methodology Code: Electrochemistry
Detection of DNA hybridization can measure the degree of similarity between two DNA sequences. Current methods for detecting DNA hybridization, while effective, tend to be slow and labor intensive. Electrochemical, folding-based DNA biosensors offer fast, sensitive and selective DNA detection. The technique electrogenerated chemiluminescence (ECL) can be used with these biosensors. These ECL-DNA biosensors combine the high sensitivity of a luminescence biosensor with the low cost and miniaturization capabilities of an electrochemical biosensor. The aim of this project is to fabricate an ECL-DNA biosensor utilizing a ruthenium complex emitter. Here, a hairpin ECL-DNA biosensor is fabricated by electrochemically cleaning an electrode, drop casting a target ss-DNA over an electrode, and allowing the electrode to sit in an alkanethiol diluent overnight, forming a mono layer. This newly fabricated ECL-DNA biosensor is then used to measure the ECL emission of the ruthenium complex in the presence of co-reactant. Next, target ss-DNA is added and the change in ECL intensity determined. This study investigated the performance and mechanism of the sensors as a function of diluent chain length and ECL coreactant identity. The stability of the sensors as a function of potential was also investigated. Sensors were utilized with both 2-(dibutylamino)ethanol (DBAE) and tripropylamine (TPrA) coreactant. TPrA-based sensors exhibited “signal-on” behavior while preliminary data shows that DBAE sensors showed “signal-off” behavior. Diluents with chain lengths of 6, 8 and 9 carbons were studied. The C9 sensors exhibited a low signal and small changes in signal upon the addition of target DNA so were eliminated from the study. It was also determined that potentials above +0.85 V versus Ag, AgCl could not be applied to the electrodes. At this point, the sensors respond to 2-micromolar additions of target DNA. Additional studies investigating sensor selectivity and linear range are ongoing.

Keywords: Bioanalytical, Chemiluminescence, Electrochemistry, Nucleic Acids
Application Code: Bioanalytical
Methodology Code: Electrochemistry
Microfluidic devices offer quick and inexpensive platforms for point-of-care clinical diagnostics. The work reported here used microfluidics to both fabricate microelectrodes and microfluidic devices for the electrochemiluminescent (ECL) detection of glucose. ECL detection methods combine the sensitivity of a luminescence method and the low cost of electrochemical methods. Carbon-paste electrodes were used bare to detect hydrogen peroxide using the luminol ECL reaction. The electrodes were characterized by cyclic voltammetry. After optimization of detection parameters such as applied potential, flow rate and luminol concentration, the electrodes were modified with glucose oxidase enzyme to fabricate biosensors. The biosensors were able to indirectly detect glucose since hydrogen peroxide is a by-product of the enzymatic oxidation of glucose. The present research has optimized the carbon paste microchip fabrication techniques, applied potential, buffer flow rate, and luminol concentration.

Keywords: Bioanalytical, Chemiluminescence, Electrochemistry
Application Code: Bioanalytical
Methodology Code: Microfluidics/Lab-on-a-Chip
A screen printing process was used to fabricate electrochemical cells with a 3 mm diameter carbon ink working electrode. A cyclic voltammetry study revealed that application of Ag ink to the reference electrode produced cells with the most stable response. The electrodes were used to produce electrogenerated chemiluminescence from solutions containing tris(2,2'-bipyridyl)ruthenium(II) and the co-reactant dibutylaminoethanol (DBAE). A mobile phone was used to image and detect the ECL signal and the software ImageJ was used to quantify the response. This detection method could differentiate between solutions over a DBAE concentration range of 0.15 mM to 15 mM. Work is underway to determine the linear range and precision of the method.
In liquid extraction techniques, sample extraction and ionization can be carried out in ambient conditions. Furthermore, these techniques can provide parallel extraction and analysis route for liquid or wet solid samples. Previous studies by our group show that nanopipettes are robust probes for selective extraction of analytes and have been used to study chemical reactions in local environments. We attempt to connect these studies and design a liquid extraction setup with nanopipettes as probes for extraction and simultaneous ionization for mass spectrometric analysis of analytes. Implementation of nanopipettes as probes to perform liquid extraction, followed by nanoelectrospray ionization through nanopipettes, can provide a platform to study chemical reactions of environmentally and biologically important samples in with high spatial resolution and sensitivity.

Keywords: Electrospray, Extraction, Mass Spectrometry
Application Code: Bioanalytical
Methodology Code: Mass Spectrometry
## Session Title
ACS ANYL Poster Session

## Abstract Title
Analysis of Products from CO[sub]2[/sub] Electroreduction Using Micro- and Nano- Probes

### Primary Author
Natasha P. Siepser  
Indiana University

### Co-Author(s)
Gargi S. Jagdale, Lane A. Baker, Soojin Jeong, Xingchen Ye

### Abstract Text
The electrochemical conversion of carbon dioxide to chemicals and fuels useful in industrial processes is an attractive approach to sequestering carbon dioxide present in the atmosphere. Commonly, product analysis following electrocatalysis requires sampling the headspace or the bulk electrolyte. However, bulk product analysis methods provide an averaged result for the entire catalyst instead of providing local product information. To address this limitation, our group is developing methods that utilize micro-/nano- size probes for studying electrochemical reaction products generated at the electrocatalyst surface at the micro/nanoscale. In this poster our most recent efforts to incorporate local chemical measurements at catalytic surfaces with scanned pipettes, specifically through scanning ion conductance microscopy, are described.

### Keywords:
Electrochemistry, Material Science, Method Development, Sampling

### Application Code:
Material Science

### Methodology Code:
Sampling and Sample Preparation
Detection of Trihalomethanes in Municipal Water and Bottled Water Using SPME GC-MS

Solid phase micro extraction (SPME) with gas chromatography mass spectrometry (GC-MS) enabled the detection of the trihalomethanes (THMs) chloroform, bromoform, dibromochloromethane and bromodichloromethane in municipal and commercially available bottled water. Data was acquired using both Scan and SIM modes of the mass spectrometer. Commercially available standards were used for quantification and determination of the limits of detection (LOD) and limits of quantification (LOQ). Noteworthy was the finding that some of the samples tested contained concentrations above the Environmental Protection Agency (EPA) maximum allowable concentrations (MAC) of 80 ppb.

Keywords: Environmental/Water, GC-MS, SPME, Water
Application Code: Environmental
Methodology Code: Gas Chromatography/Mass Spectrometry
A 3D-Printed Equilibrium Dialyzer with Plate Reader Compatibility

Measuring the binding affinity of small biological ligands to macromolecules, such as plasma proteins, is an important quantitative determination in life science research, as it helps scientists understand the bioavailability of the ligand to cells and tissues in the body. Equilibrium dialysis is the most common technique for this measurement, enabling the separation and subsequent determination of the free and bound ligand, which can then be used to calculate a binding constant. Traditionally, this technique is labor intensive, time consuming, and requires multiple steps to make a single accurate measurement of binding affinity. Here, we present an equilibrium dialysis device fabricated by a 3D printer with the same dimensions as a standard 96-well plate, thereby enabling measurement of the free ligand of 12 samples, simultaneously, with a plate reader without transferring any of the samples after equilibrium has been established. While two equilibrium dialyzers amenable to 96-well plate technology are commercially available, both are limited to only a couple of membrane materials and molecular weight cutoff values. The system reported here, due to a novel print-pause-print technique used during the course of additive manufacturing of the device, enables the user to employ custom membrane materials and control variables such as pore size. The device was characterized by measuring the binding affinity of fluorescein to physiological concentrations of albumin (42 g/L) to calculate a Kd and a %PPB (percent plasma protein bound) of 7.2 ± 1.3 × 10⁻⁵ M and 81.3 ± 2.8 %, respectively, with the utilization of a 3.5 KDa MWCO regenerated cellulose membrane. Both values are comparable to those reported in the literature. The device is capable of rapidly and accurately measuring the binding affinity of ligands detectable by fluorescence, absorbance, and liquid scintillation counting.

Keywords: Bioanalytical, Lab-on-a-Chip/Microfluidics, Membrane, Pharmaceutical
Application Code: Pharmaceutical
Methodology Code: Microfluidics/Lab-on-a-Chip
A Versatile Platform for Pressure- or Diffusion-Driven Membrane-Based Separations

Membrane based separations, including ultrafiltration and membrane affinity chromatography, are often limited by such properties of commercially available membranes as pore size, pore density, and material. Here, we present a simple device creation method involving additive manufacturing that enables any membrane to be embedded into a 3D printed O-ring via a print-pause-print approach. This approach creates a leak-free seal around the membrane that can withstand relatively high amounts of pressure. This embedded membrane can then be placed in a 3D printed holder designed to couple directly to a syringe, or to tubing for coupling with a syringe or peristaltic-type pump, for delivery of sample through the pores for the execution of complex membrane-based separations. The device described here was used to measure the binding affinity between small ligands and large serum proteins. The device was characterized by utilizing a 3.5- kDa cut off dialysis membrane to separate 100 μL of free fluorescein from 1 mL of an albumin fluorescein mixture. This yielded a dissociation constant of 236 +/- 6 μM, a value within reported literature values. For this experiment, a syringe pump was utilized to drive reagents through the pores and achieved flow rates up to 1.5 mL per hour without bursting the membrane. This same device was utilized to measure important biological metals (Zn) binding to albumin. Finally, when the membrane pores are modified, the system can be used to perform affinity-based separations with the same simplicity and speed as unmodified membranes.

Keywords: Biotechnology, Membrane, Protein, Separation Sciences
Application Code: Bioanalytical
Methodology Code: Separation Sciences
The rise of antibiotic resistant bacteria has necessitated faster diagnostic tools and novel antibiotics. Increasing requirements to create FDA-approved drugs, coupled with the threat of bacteria evolving antibiotic resistance to those drugs, has led to fewer new antibiotics on the market. Here we present a device to determine bacterial-antibiotic susceptibility. The device imitates the pharmacokinetics of a drug in vivo. Key to the device is a printed cubical insert that contains 2 membranes dividing the device into 2 diffusion-based compartments. The printed cube is printed as a flat, 2-dimensional piece with “hinges” that can be folded up into a cubical, 3-dimensional insert that is placed into a fluidic device. To perform antibiotic resistance measurements, bacteria are grown to logarithmic growth phase and then transferred to the device. A buffer containing antibiotic is pumped beneath the central-compartment allowing diffusion of the antibiotic to the central-compartment (representing absorption of the drug in vivo) and then the peripheral-compartment (representing distribution). Next, the antibiotic concentration gradient is reversed by flowing buffer with no antibiotic (representing excretion). This delivery scheme enables a PK-type profile to be delivered to the bacteria, similar to a patient taking a daily oral dose of antibiotic. To quantitatively determine the effect of the drug on bacterial death, the ratio of ATP released by the bacteria to the bacterial turbidity (OD600) is measured at various intervals. The peak point of ATP/OD600 ratio is indicative of antibiotic efficacy against a bacterial strain. To date, we have demonstrated this method works for drugs against gram negative and gram positive bacteria and that bacterial efficacy can be determined in as little as 20 minutes, an improvement over standard methods, such as disk diffusion rings, which are qualitative and require overnight incubation between the drug and the bacteria.

**Keywords:** Bioanalytical, Lab-on-a-Chip/Microfluidics, Pharmaceutical, UV-VIS Absorbance/Luminescence

**Application Code:** Pharmaceutical

**Methodology Code:** Microfluidics/Lab-on-a-Chip
Leptin, a hormone produced mainly by adipose tissue, is believed to balance energy levels in vivo by regulation of food intake and body weight. Higher concentrations of leptin are observed in obese people, who are thought to be “leptin resistant”. Obese diabetic patients do not exhibit a strong response to exogenous leptin thus a detailed understanding of the glucose regulatory function of leptin is of importance to overcome the shortcomings of leptin therapy in humans. Previously, our group has reported that C-peptide, a 31 amino acid peptide secreted from pancreatic beta cells, binds to red blood cells (RBCs) and has cellular energetic effects. Here, we show that leptin actually amplifies the effects of C-peptide. ATP release from RBCs was measured in the presence of leptin, C-peptide, zinc, and combinations thereof, while also monitoring the translocation of GLUT-1 to the RBC membrane. In the presence of C-peptide and zinc, a 56 ± 1% increase in RBC-derived ATP is measured; this signal is enhanced by another (27 ± 2) % in the presence of leptin. Interestingly, leptin in the presence of zinc and C-peptide increased GLUT-1 translocation by (21 ± 3) % compared to the control and samples including zinc and C-peptide only. To mimic in vivo circulation, a 3D printed microfluidic device has been used to determine ATP released from RBCs in the presence and absence of leptin when INS-1 cells are deposited on the membrane of an insert to mimic pancreatic cells secretion. Our studies show that levels of leptin in the bloodstream may enhance the cellular effect of C-peptide. Therefore, leptin along with zinc and C-peptide can potentially be targeted as a diabetes therapeutic.
Abstract Text

Students find it more exciting to analyze real-world samples and they develop a better recognition for analytical methods used to determine the quantity of an analyte in such samples. In an effort to make the analytical chemistry laboratory more relevant to real-world problems and situations, the authors chose to incorporate a pharmacy-chemistry interdisciplinary lab focused on analyzing the strength of compounded electrolyte IV admixture solutions. The experiment presents students with the opportunity to evaluate the feasibility of using United States Pharmacopeia (USP) methods to assess the quality of compounded sterile preparations (CSPs) prepared by pharmacy students. USP titration methods of calcium gluconate and magnesium sulfate were implemented using selected IV admixture bags prepared by pharmacy students and the final strength of the electrolyte IV admixture bags were determined. Students gave positive feedback for this real-world analysis with this being the most-liked part of the lab.

Keywords: Education, Pharmaceutical, Quality Control, Titration
Application Code: Pharmaceutical
Methodology Code: Education/Teaching
Abstract Text

Opiate dependence is a potentially life-threatening disorder associated with adverse physical and societal effects. In the United States it is currently reaching the level of epidemic. Because of relatively low amount of active pharmaceutical ingredient (API) in many of opiate formulations, its unambiguous determination could be problematic. To complicate the issue, optical isomers of most compounds of interest have dramatically different physiological properties.

Here we present our results on determination of common morphinan-type compounds (dextromethorphan, naltrexone, etc.) in various preparations. To address the problem, we extracted API using standard Pharmacopoeia procedures. Resulting solutions were examined using FTIR, GC-MS (with and without appropriate derivatization) and NMR techniques. We also suggest that easy-to-grow crystals of alkaloid metal complexes or solvates may provide a suitable analytical approach for unambiguous identification using single crystal X-ray diffractometry. Current state of instrumentation allows use of crystals with dimensions less than 0.05 mm along with data collection/processing time being less than 10 min per sample. All the mentioned experimental data combined provide practically unambiguous identification of API as well as necessary information about formulation matrix and admixtures. Only sub-mg quantities of API were necessary. Such comprehensive approach serves as a valuable training tool in education of analytical chemists working with drug determination problems.

Financial support from the State University of New York for acquisition and maintenance of the X-ray and MS instrumentation is gratefully acknowledged.

Keywords:  Chiral, Identification, Pharmaceutical, X-ray Diffraction
Application Code:  Pharmaceutical
Methodology Code:  Education/Teaching
The production and use of graphene family nanomaterials (GFN) have raised concerns about the potential release of GFN into the environment. Like graphene, graphene oxide (GO) exists as 2-dimensional sheets, but GO is much more readily dispersed in water due to the presence of edge carboxyl groups as well as hydroxyl and epoxy groups in the basal plane. Studies have shown that GO may be transformed under sunlight in aqueous media to yield both reduced GO products (rGO) as well as oxygenated PAHs. In this work we report on the effects of sunlight on GO and rGO particle size, charge, and morphology using dynamic light scattering and atomic force microscopy. Carboxylated and hydroxylated PAH photoproducts are identified and quantified with LC/MS-MS using neutral loss scanning and multiple reaction monitoring modes. Key properties for simulating the environmental fate of the oxidized PAHs (Koc, pKa) are calculated using physical-chemical property estimation techniques, and formation rates of GO photoproducts are reported and discussed in the context of simulating GO environmental fate in surface waters.
A new experiment related to food science was designed and validated to use as a standard laboratory procedure for teaching quantitative analysis course. Pretzels from grocery store was used as the sample and their sodium content was determined by using an ion selective electrode. Advantage of using a packaged food sample is that the sodium content printed on the package can be used to compare with the student's results. The experimental results show a linear calibration curve for a three order of magnitude in sodium concentration ranging from 0.1 to 0.0001 M of sodium ions. Interestingly and as expected, the determined sodium content has been lower than the number which was labelled on the package. This may be due to the smaller sampling size used for the analysis that the sodium content printed on the label. From this experiment, students are able to learn the importance of sampling, sample treatment, and method validation. A sodium recovery study was performed by adding sodium standards to the pretzel samples and the recover was found 99.2% from three samples.
The study deals with the synthesis and characterization of a series of biologically significant new mononuclear palladium(II) complexes of benzohydrazides and pyridinecarbohydrazides. All the compounds were subjected to high throughput screening in order to find most potential palladium-based inhibitors of DPPH free radical. In order to perform the mechanistic studies of DPPH scavenging by Pd(II)-hydrazide complexes, UV-visible spectroscopy was utilized. UV-visible spectral study indicates a two step mechanism for the reaction of DPPH inhibition: an electron transfer to DPPH from Pd(II)-hydrazide yielding DPPH\(^{-}\) followed by a proton transfer to form DPPH-H. In addition, a preliminary structure activity relationship (SAR) study was executed for optimization of structure to enhance biological activity for future applications. To elucidate the chemical structures of Pd(II) complexes, various methods were adapted, which comprise spectroscopic techniques (\(^{1}\)H-NMR, \(^{13}\)C-NMR and FT-IR), CHN analysis, metal content determination, and conductometric measurements. The pyridinecarbohydrazide ligands revealed to act as bidentate N,O donors coordinating through amino nitrogen and carbonyl oxygen, producing 1:1 metal-ligand complexes of type [PdLCl\(_2\)]. In contrast, the benzohydrazides were likely to show monodentate binding mode (as N donors), yielding 1:2 metal-ligand complexes of general formula [PdL\(_2\)Cl\(_2\)], for which the metal coordinating site was amino nitrogen. All Pd(II) hydrazide complexes were found to be considerably more potent inhibitors of DPPH free radical compared to free hydrazide ligands. These complexes are even stronger DPPH scavengers than standard antioxidant propyl gallate. Interesting mechanistic study has been evaluated.

**Keywords:** Characterization, Drug Discovery, Metals, UV-VIS Absorbance/Luminescence

**Application Code:** Drug Discovery

**Methodology Code:** UV/VIS
In today's world, a Bachelor's degree chemist is expected to not only operate numerous types of instruments but they also must be able to troubleshoot them, develop new methodologies, collaborate in teams, and report on results. Therefore, it is essential for students to get away from treating instruments as magic black boxes. Presented here is a guided inquiry fluorescence experiment that allows students to truly understand the origin of their fluorescence data. Several basic principles of fluorescence are covered including integration time, sample averaging, excitation light intensity, concentration dependence, inner filter effect, measuring an unknown, varying pH and quenching.

**Keywords:** Fluorescence, Spectroscopy, Teaching/Education, UV-VIS Absorbance/Luminescence

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**Co-Author(s):**

Melissa Hill

Vernier Software & Technology
A Portable GC/PID for the Analysis of Odorous Sulfur Compounds in Ambient Air

A capillary column gas chromatography method was used for the analysis of sulfur compounds such as hydrogen sulfide, methyl mercaptan, dimethyl sulfide, and dimethyl disulfide in ambient air. The concentration of these sulfur compounds is typically very low (low to sub ppb) in ambient air. Their odor threshold is at the low ppb levels so it is also necessary to make field measurements near odorous sulfur sources such as refineries, pulp and paper mills, etc. because of the high reactivity of the sulfur compounds. A portable gas chromatograph (GC) with a photoionization detector (PID) and concentrator is ideal for this application because of the high sensitivity of the PID to sulfur compounds and it does not need support gases such as zero air or hydrogen. It has more than 100 times the sensitivity of the flame photometric detector and a much wider dynamic range.

Keywords: Air, GC, Industrial Hygiene, Portable Instruments
A new analytical method has been developed for the analysis of octreotide in mouse plasma using liquid chromatography tandem mass spectrometry. Octreotide is a 1018 Da octapeptide that mimics somatostatin and has been shown to be useful in reducing radiation-induced intestinal damage. This method involves simple sample preparation using weak cation exchange solid phase extraction followed by evaporation and reconstitution in 20% acetonitrile. Analytical separation was performed on a YMC-Pack-ODS-AQ analytical column (150 x 3.0 mm (i.d.) with a chromatographic run time of 5.00 minutes. In developing this method two different internal standards, leuprolide, and Octreotide-D5, were evaluated for matrix ion effects, and extraction recovery. Using leuprolide as an internal standard showed significant matrix ion enhancement of 119±59.9% and an extraction recovery of 70±10.5%. By using the stable isotope octreotide-D5 as an internal standard, matrix ion effects were negligible at -5.0±6.5% and extraction recovery was 88±8.3%, this lead to an overall process recovery of 84±7.8%. The lower limit of quantitation (LLOQ) was determined to be 10 ng/mL based on a signal to noise ratio of 5, and the lowest concentration that maintained acceptable accuracy and precision defined by the FDA’s Guidance for Industry for Bioanalytical Method Validation. Accuracy for 5 calibration standard sets were between 87% and 114% based on a 1/x weighted linear regression. Intraday precision was $\pm$17% at the LLOQ, and $\pm$10% at non-LLOQ concentrations and interday precision was $\pm$19% at LLOQ and $\pm$11% at non-LLOQ concentrations. Freeze-thaw stability was tested at concentrations of 10 ng/mL, 100 ng/mL, and 300 ng/mL and were shown to be stable for three freeze-thaw cycles. Also three sets of calibrations standards were analyzed and shown to be stable at 4°C for at least 24 hours. Octreotide was quantitated in 5 authentic mouse plasma pharmacokinetic samples to further validate this method.
Complex mixtures, such as those encountered in biology, are often analyzed by LC-MS because of the tremendous resolving power of combining these techniques. Powerful LC-MS platforms have become the norm for metabolomics for example. Even though mass spectrometers are providing ever higher resolution, improved separations are often found to improve the detection of metabolites by reducing ionization suppression and simplifying spectra interpretation. Improving the resolution of HPLC can be achieved by using smaller particles or longer columns. Both approaches require higher pressure for driving mobile phase flow so that pressure available can be considered a limiting factor in resolution. Even with high resolution, the broad polarity range of metabolomics samples makes it difficult to resolve all compounds in a mixture. In this work we describe use of an HPLC system capable of generating 40,000 psi for analysis of metabolomic mixtures. We also describe derivatization strategies that improve separation and detection of polar compounds when using reversed phase columns with ESI-MS.

Keywords: Bioanalytical, Biological Samples, Liquid Chromatography/Mass Spectroscopy

Application Code: Bioanalytical

Methodology Code: Liquid Chromatography/Mass Spectrometry
**Abstract Title**: A Bonded Phase for Resolution and Sensitivity in RPLC-MS of Proteins

**Abstract Text**

Historically there has been a tradeoff between LC resolution and MS sensitivity for protein analysis. RPLC has given the best resolution when trifluoroacetic acid is used as an additive, but this additive markedly reduces sensitivity for electrospray ionization. Formic acid, on the other hand, allows for high sensitivity with electrospray ionization, but the resolution in RPLC suffers due to charge interactions between the protein and the silica surface. A new class of bonded phases for RPLC is presented here that preserves the LC resolution with formic acid as an additive. These bonded phases are made by growing polymer chains on silica by atom transfer radical polymerization. The thickness of the bonded phase is greater than the Debye length, thereby reducing the charge interactions with the surface, while the hydrophobicity of the polymer provides the reverse-phase selectivity. The performance of the bonded phase in RPLC-MS is evaluated using commercial monoclonal antibodies, as well as pharmaceutical monoclonal antibodies and antibody-drug conjugates.

**Keywords**: Bioanalytical, Biopharmaceutical, Liquid Chromatography, Liquid Chromatography/Mass Spectrometry

**Application Code**: Pharmaceutical

**Methodology Code**: Liquid Chromatography/Mass Spectrometry
Nano-flow LC is desirable for applications that require very low consumption of mobile phase and light weight/portable instrumentation. Among these are process stream, clinical and field analyses. Fused silica capillary columns with diameters of 150 microns packed with 3 micron diameter particles typically require less than 500 nL/min flow rates at reasonable pressures. Compact LC systems have recently been developed that can produce highly accurate nL flow rates under gradient programming conditions without solvent splitting, and light-emitting diodes (LEDs) are becoming increasingly available for desirable analytical wavelengths. We recently found that UV absorbance measurements at multiple wavelengths, combined with chromatographic retention data from multiple stationary phase segments arranged in series in a single column can be used to provide simple and reliable target analyte identification. Small LED detectors designed for measuring absorptions on-column at selected wavelengths can be placed at strategic positions along a multi-segment column to provide the necessary data for compound detection. Clever approaches are required to prepare capillary columns for multi-point on-column detection, since a UV transparent window through the capillary is required after each packed segment. Information obtained from multi-wavelength absorption ratios can be combined with retention data to greatly magnify the “detection capacity” for target analytes.

Keywords: Capillary LC, Environmental, HPLC Detection, Portable Instruments
Application Code: Environmental
Methodology Code: Liquid Chromatography
Universal Derivatization of Metabolites for Improved Sensitivity in LC-MS

Introduction: Metabolomics, the study of small molecules involved in cellular processes, offers the potential for investigating disease pathogenesis. Electrospray ionization mass spectrometry (ESI-MS) is widely used in metabolomics due to its high sensitivity and ability to generate qualitative information. Metabolites are a diverse group of compounds with a variety of functionalities including hydroxyl, amine, carboxyl, phosphoryl, and thiol groups. However, the structural diversity of metabolites results in differential signal response with LC-ESI-MS and consequently varying degrees of sensitivity. These limitations hinder detection of molecules.

Methods: This work undertakes a derivatization approach to improve electrospray by tagging multiple functional groups to boost metabolite sensitivity. By derivatizing most functional groups with high proton affinity tags, adduct formation and in-source fragmentation is dramatically diminished. Using two distinct tags to label hydroxyl, amine, carboxyl, phosphoryl, and thiol groups on various metabolites, diverse metabolites can be analyzed in a single run. Separation of tagged metabolites has proven a complex challenge and RP, HILIC, mixed-mode, capillary electrophoresis and capillary LC will be discussed.

Preliminary Data: Detection of diverse metabolites (sugars, amino acids, alcohols, and carboxylic acids) are analyzed in a single chromatographic run. Limits of detection for these compounds are in the low nM range. This method has been applied to cell culture to show the potential to analyze complex samples.

Novel Aspect: This work shows the potential to simultaneously separate and analyze diverse metabolites using a simple two step tagging procedure.

Keywords: Amino Acids, Bioanalytical, Derivatization, Liquid Chromatography/Mass Spectroscopy

Application Code: Bioanalytical

Methodology Code: Liquid Chromatography/Mass Spectrometry
Protein glycosylation is a prevalent, chemically complex, and biologically diverse post-translational modification (PTM) involved in a wide array of intra- and inter-cellular functions. Approximately half of all expressed proteins undergo glycosylation, and this heterogeneous modification accounts for the greatest proteome diversity over any other PTM. Changes in protein glycosylation are associated with cellular proliferation, inter-cellular communication, and metabolic processes, making the characterization of the cellular landscape of protein glycosylation integral to advancing our understanding of cell biology. Glycan microheterogeneity, i.e., different glycans modifying the same glycosite, makes glycan identity at a given site crucial to the biological context of the modification. Tandem mass spectrometry (MS) is an ideal platform to advance glycoproteomic technology, but current dissociation methods are often suitable only for characterization of either peptide or glycan moieties. We have developed a tandem MS dissociation method called activated ion-electron transfer dissociation (AI-ETD) that addresses several of the challenges of intact glycopeptide analysis. Through the use of concurrent ion-ion reactions and infrared photo-activation, AI-ETD can access glycan and peptide information from intact glycopeptides in a single MS/MS scan, and with this AI-ETD provides (1) improved product ion generation for peptide backbone sequencing, (2) higher MS/MS success rates to sequence more glycopeptides per experiment, and (3) valuable fragmentation for glycan composition determination. Here we showcase AI-ETD for large-scale intact glycopeptide characterization on the newest generation of Orbitrap instruments. We show that >2000 localized N-glycosites can be confidently identified from approximately 30,000 localized N-linked glycopeptide spectral matches (>7,500 unique) in mouse brain tissue.

Keywords: Capillary LC, HPLC, Ion Trap, Tandem Mass Spec
Application Code: Genomics, Proteomics and Other 'Omics
Methodology Code: Mass Spectrometry
Malfunctions of the brain’s serotonin system are thought to underlie the symptoms of depression, primarily because antidepressants that target the serotonin transporters show some clinical success. However, antidepressant efficacy is variable, often delayed and temporary. Because the roles of serotonin in the brain during health and disease are ill-defined, it is extremely challenging for drug developers to develop better, more targeted antidepressant therapies. This situation is confounded because current therapies assume that the serotonin system dysfunctions in the same way regardless of the multitude of causes (psychological, genetic, exposure, disease) of human depression. In this work, we develop and apply novel voltammetric tools and couple them with confocal imaging, animal behavior and mathematical models to characterize serotonin neurotransmission in different animal models of depression. Specifically we find that alterations in another neurotransmitter system, the histamine system, likely modulate abnormalities in the serotonin system. Additionally we find that rapid changes in serotonin transporter function may be responsible for the dosing and delayed therapeutic issues of common antidepressants. We model the experimental data and offer mechanistic insights into how histamine and serotonin modulate one another in health and disease and this leads us to hypothesize why antidepressants are not universally effective. The data afforded by our chemical tools has the capacity to profoundly improve therapeutic strategies towards depression by shedding light on another important player.

**Keywords:** Bioanalytical, Monitoring, Neurochemistry, Voltammetry

**Application Code:** Neurochemistry

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

Each year in the US 1.7 million people suffer a traumatic brain injury (TBI) of whom 52,000 die, 275,000 are hospitalised and 1.39 million are treated in the emergency room before release [1]. TBI contributes to 31% of all injury related deaths in the US.

These stark statistics prompted us to begin a journey to adapt our experience from neurochemical monitoring in experimental brain models to the very challenging task of monitoring human traumatic brain injury patients in the intensive care unit. Our goals were (1) to give the clinical care team moment by moment information about the brain health, (2) to understand the mechanisms leading to the deterioration of 60% of severe TBI patients many days after admission to the intensive therapy unit. A key part of my group at this time was a bright-eyed, super-smart graduate student called Parry Hashemi.

This talk will describe recent advances in on-line monitoring of multiple neurochemical analytes by use of microelectrode sensors and biosensors within 3D printed microfluidic devices. The talk will also demonstrate how new technologies such as FET array detectors and droplet microfluidics can increase the resolution of small concentration changes and dynamic response of the assay system.


**Keywords:** Biosensors, Electrochemistry, Lab-on-a-Chip/Microfluidics, Neurochemistry

**Application Code:** Neurochemistry

**Methodology Code:** Microfluidics/Lab-on-a-Chip
The intestinal tract is responsible for processing food to extract key nutrients and water and remove unwanted waste. One of the key approaches that the intestine conducts these actions is through motility. One key layer of the intestinal tract is the mucosa, which acts as the interface between the lumen (where the food passes) and the enteric nervous system, which coordinates the smooth muscle of the intestines. Therefore, the signalling molecules within the mucosa act as key transducers. These signalling molecules in recent years have been of great interest due to their role in driving motility, altering the inflammatory status of the bowel and communicating the microbiota. Of the signalling molecules investigated, serotonin is by far the most investigated and has been shown to be a key player in driving function, however its role in motility is still contested. Another molecule, melatonin has recently been shown to be present within the mucosa and little is known about its functional activities.

Much of the limitation on understanding these molecules span to the difficult measurement environment and the challenges on developing sensor devices that can suit the dynamic nature of intestinal muscle movements. This presentation will showcase new sensor approaches to understand signalling and muscle dynamics and provide insight into how these alter with disease.

Abstract Text

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Keywords: Bioanalytical, Biomedical, Electrochemistry, Microelectrode
Application Code: Biomedical
Methodology Code: Electrochemistry
Microdialysis (MD) sampling is an in vivo sampling technique that makes it possible to continuously monitor substances in the extracellular fluid of the brain. The amount of analyte recovered by microdialysis sampling is a function of the flow rate used. Therefore, to obtain the necessary temporal resolution for many neurotransmitters of interest, a highly sensitive method capable of analyzing submicroliter volumes is desirable. Microchip electrophoresis (ME) is an analytical method that can analyze submicroliter samples, with typical analysis times of 5-90 seconds. The on-line coupling of microdialysis with microchip electrophoresis yields a “separation-based sensor” that can continuously monitor multiple neurotransmitters with good temporal resolution. Progress toward the development of unique MD-ME systems for catecholamines, adenosine metabolites and amino acids will be described.

Keywords: Electrochemistry, Fluorescence, Lab-on-a-Chip/Microfluidics, Neurochemistry
Application Code: Bioanalytical
Methodology Code: Microfluidics/Lab-on-a-Chip
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<td>Abstract Title</td>
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<tr>
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<td>University of Pittsburgh</td>
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<td>Co-Author(s)</td>
<td>Andrea Jaquins-Gerstl, Elaine M. Robbins</td>
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**Abstract Text**

Microdialysis has been used to obtain samples of the extracellular fluid of the brain in animals and patients since the 1970s. The benefits of microdialysis for in vivo chemical monitoring are numerous and include its wide scope, its ability to produce samples free of cellular debris, its compatibility with numerous animals species, the absence of undue surgical complications, and so on. Nevertheless, it remains true that implantation of microdialysis probes into brain tissue causes a penetration injury that, in turn, triggers a wound-healing response that, left unattended, leads to encapsulation of the probe in a glial scar. Once the scar is formed, the probes, while remaining fully functional in and of themselves, lose contact with the surrounding tissues. Thus, the probes report scar-derived chemistry of limited information content. To address this point, we have pursued “dexamethasone enhanced microdialysis.” Retrodialysis of dexamethasone, a powerful anti-inflammatory drug, has proven highly effective at preventing gliosis at the probe site and preserving blood flow and protecting cells, neurons, and terminals. With dexamethasone enhancement, probes remain functional in the brain for at least 10 days, the longest recording yet attempted. Ongoing studies are aimed at dexamethasone enhanced microdialysis for monitoring glucose and potassium ion for 10 days in a rodent model of traumatic brain injury.

**Keywords:** Biosensors, Microelectrode, Monitoring, Neurochemistry  
**Application Code:** Bioanalytical  
**Methodology Code:** Sensors
Traces of body fluids discovered at a crime scene are a potential source of DNA, which is a major individual evidence in the modern forensic investigation. We have recently reported on the application of Raman spectroscopy for nondestructive, confirmatory identification of biological stains at a crime scene including dry traces of sweat, vaginal fluid, semen, saliva, and blood. The method allowed for differentiating animal and human blood as well menstrual and peripheral blood. Most recently, the method was further developed for determining the time since deposition for bloodstains for up to two years. It would be of great help for criminal investigation to develop a phenotype profiling immediately at a crime scene based on a rapid analysis of biological stains. With this goal in mind, the possibility of race differentiation based on Raman spectroscopy of blood traces was investigated. Specifically, advanced statistical analysis of spectroscopic data was used to discriminate between Caucasian and African American donors based on dry peripheral blood traces with over 80% accuracy. This initial work was followed by further proof-of-concept studies demonstrating the differentiation of donor’s sex based on bloodstains and saliva traces as well race differentiation based on traces of semen. Overall, the developed method has a great potential for crime scene investigation, providing rapid and reliable results, with no sample preparation, destruction, or consumption.

This project was supported by Award No. 2014-DN-BX-K016 awarded by the National Institute of Justice, Office of Justice Programs, U.S. Department of Justice. The opinions, findings, and conclusions or recommendations expressed in this publication are those of the authors and do not necessarily reflect those of the Department of Justice.

Keywords: Chemometrics, Forensics, Raman Spectroscopy, Vibrational Spectroscopy
Application Code: Homeland Security/Forensics
Methodology Code: Vibrational Spectroscopy
Advances in Raman Spectroscopy

Electrochemical Raman Spectroscopy at Time and Space Limits

Surfaces and interfaces play a key role in heterogeneous reactions. The electronic and geometric structure of the surface may significantly influence the surface reactions and the surface active sites are considered to play the most important role. Therefore, it is extremely important to develop a method to probe the surface structure and the interaction of the reactant or product with the active sites. In addressing the spatial resolution, tip-enhanced Raman spectroscopy (TERS) appears to be an ideal tool. We demonstrated that TERS can chemically and spatially resolve the site-specific electronic and catalytic properties bimetallic model catalysts of Pd or Pt on Au(111) at 3 nm resolution, with vibrational fingerprints of phenyl isocyanide (PIC) adsorbed and reacted on the surface. We further developed electrochemical TERS (EC-TERS) method and applied it to synergistically control the reaction by both electrode potential and laser power and characterize the reaction with nanometer spatial resolution. The plasmon-induced reaction can lead to a reaction region of 30 nm in radius, which equals the mean free path of Au. Such a high spatial resolution.

Furthermore, the electrochemical interfaces are highly dynamic changing systems, which should be investigated under potential control and presence of the electrolyte. The conventional electrochemical methods do not have the capacity of providing both time and spatial resolution. To address this problem, we developed electrochemical surface enhanced Raman microscopy (EC-SERM), which allows us to study the highly dynamic reacting system at the same time scale as the electrochemical control, typically in the millisecond, over the whole surface. In this way, we are able to monitor the spatial distribution of electrochemical activity over the electrode surface at the same time, which may have important impact for electrochemistry and energy and corrosion-related studies.

Acknowledgement: We thank supports from MOST & NSFC.

Keywords: Adsorption, Electrochemistry, Spectroelectrochemistry, Surface Enhanced Raman Spectroscopy

Application Code: Nanotechnology

Methodology Code: Vibrational Spectroscopy
Nonlinear Raman imaging, specifically stimulated Raman scattering microscopy (SRSM), is emerging as a useful imaging method in biomedical analysis since it combines advantages of traditional Raman microscopy and addresses limitations of speed and spatial resolution. However, SRSM allows for probing specific discrete molecular vibrational modes instead of the entire spectrum. Here we discuss the trade-offs in using the same for biomedical imaging and histopathology. As examples, we present applications of SRSM towards classification of different brain cell types and tissue variations in prostate cancer. Specific morphological regions of the brain were recorded using spontaneous Raman spectroscopy and imaged using SRSM. We were able to segment tissue into different regions and provide visualizations comparable to optical microscopy. By using statistical pattern recognition to relate spectral intensities to conventional color in stained histologic images, we could provide high quality stainless images that resembled those used for clinical and research purposes. Similar analysis in prostate tissue can provide high quality images of changes in both epithelial tumor cells and the surrounding tumor microenvironment.

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**Keywords:**  Microscopy, Raman Spectroscopy, Spectroscopy, Vibrational Spectroscopy

**Application Code:**  Biomedical

**Methodology Code:**  Vibrational Spectroscopy
Although the Raman effect is a weak light-matter interaction, strong signals can nonetheless be generated with the help of the enhanced optical properties of nano-structured metals. The concentration of the excitation field and the enhancement of radiative rates mediated by the metal’s plasmonic modes can raise the otherwise imperceptible Raman signal from single molecules to detectable levels. The amplifying qualities of metallic nano-structures are ideally suited for detecting and identifying molecular species at low concentration, spurring the development of chemical sensors based on surface-enhanced Raman scattering (SERS) into a burgeoning field. Translation of these principles to nonlinear Raman techniques, however, has not been trivial. The different heating kinetics under pulsed illumination, combined with nonlinear optical radiation from the metallic antenna itself, has complicated the realization of clean and reproducible nonlinear Raman experiments. Nonetheless, various efforts have shown potential. In this contribution, we discuss various realizations of surface-enhanced coherent anti-Stokes Raman scattering (SE-CARS) experiments. We start with SE-CARS on flat gold surfaces, followed by experiments on nano-antennas in the single molecule limit. We study the relevant mechanisms at play and discuss the general implications of surface-enhanced coherent Raman techniques.
Deep-ultraviolet Raman spectroscopy is a very useful approach for standoff detection of bulk and trace explosives. The combination of large Raman signal strengths and negligible background from deep-ultraviolet excitation should allow for high-specificity and high-sensitivity in detection of explosives from a standoff distance. The High Technology Foundation has been evolving two prototype explosive detectors based on resonance Raman spectroscopy. The first system was enabled by small, portable deep ultraviolet sources at 236.5 nm and 257.5 nm excitation wavelengths with powers of approximately 2 mw each, and a compact dual band Raman spectrometer covering 242 nm to 272 nm range with a resolution of 30 cm⁻¹. Solid, bulk explosives were identified or detected from distances varying from 1 m up to 15.3 m. Measurements were performed on trace explosives from a distance of 2 m. Receiver operating characteristics were calculated from large number of measurements to assess the utility of the underlying techniques and the detector. Ammonium perchlorate was detected with a true-positive-rate of greater than 90%, and a false-positive-rate of less than 0.2% even at a small signal-to-noise (SNR) ratio of 1.6 using the dual-excitation detector. To achieve a similar performance using a single-excitation-wavelength detector, consisting of 30 cm⁻¹ resolution spectrometer, an SNR of approximately 10 was needed. This talk will also describe the second detection system currently in development for field use. This system is compact and uses a single excitation wavelength at 236.5 nm.

Keywords: Laser, Raman Spectroscopy, Sensors
Application Code: Homeland Security/Forensics
Methodology Code: Portable Instruments
After nearly a century of misinformation and stigma surrounding cannabis plants, there is a great need for stronger interaction amongst cannabis industry experts, analytical scientists, medical professionals, patients, regulators, and teachers. We must bridge the gap between analytical sciences and the medical cannabis industry to help improve the quality of medicinal cannabis products being used by patients. This presentation will take a closer look at specific, recent activities to educate, empower and inform, including: articles, conferences, symposia, conferee networking sessions and “Canna Boot Camp” hands-on workshops for a new cross-disciplinary perspective.

While these efforts help lay a solid foundation for information sharing, we have a very long way to go towards the development of courses incorporating new pedagogical cannabis approaches, including the revision of existing medical curricula and the development of courses bridging cannabis and teacher education. We will examine the driving forces that promote cannabis change and the restraining forces.

The current landscape of cannabis testing is improving, but it is fragmented and proprietary. Cannabis labs use different analytical instrumentation and methods, as well as different sample preparation techniques. Pesticide lists vary from state to state, if testing is required at all. Federal laws prohibit labs from working with cannabinoids standards above concentrations of 1 mg/ml. Testing rules and regulations vary from state to state, further complicating matters. Cannabis classification and standardization (on many levels) are slowly improving, but we must catalyze these efforts. We must work together to move beyond regional or even national standardization toward international cannabis standards and methods.
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Co-Authors

Abstract Text

As Cannabis becomes a mainstream product in the United States, it’s important to protect consumers from unhealthy or harmful products. Information about the potency and purity of Cannabis samples and Edibles needs to be reliable. Sample Preparation of these products is essential to Cannabis testing and can ensure accurate results. But how do you prepare your Cannabis and edible samples for testing? This presentation will discuss why you need to prepare your samples and the best tools you can use to do it. We will also discuss the different methods that you can use to prepare your samples for potency and pesticide testing. Understanding the fundamentals of Sample preparation will help you to make informed decisions so that you can get the best results.

Keywords: Consumer Products, Food Safety, Pesticides, Quantitative

Application Code: Other

Methodology Code: Sampling and Sample Preparation
Quality control and labeling of cannabis varies from state to state and country to country. So whether it is protecting brand name, providing customers with more information, or educational research, a number of analytical tools are utilized. As a youngster my father told me tools are 90% of the job. The same applies to cannabis testing. The tools or analytical instruments depend on the testing to be completed, identified, and quantified. Testing includes potency profiling, terpene profiling, contaminate testing for pesticides, residual solvents, heavy metals, mycotoxins/aflatoxins, moisture content, and medical research. This seminar will discuss which analytical instruments are required for testing each of these parameters.

Keywords: GC-MS, HPLC, ICP-MS, Liquid Chromatography/Mass Spectroscopy
Application Code: Bioanalytical
Methodology Code: Liquid Chromatography/Mass Spectrometry
Potency testing and quantification of cannabinoids in cannabis and products derived from cannabis and hemp extracts is required by every U.S. state where cannabis is legal for either recreational or medicinal use. Oils extracted from hemp include cannabinoids such as cannabidiol (CBD), cannabidiolic acid (CDBA), cannabiol (CBN), and cannabigernol (CBG) in relatively high to moderate concentrations in the plant material, and are non-psychoactive (Δ9-tetrahydrocannabinol (THC) less than 0.3 % by weight). In this study, we used UHPLC and mass spectrometry to determine potency, and quantitatively profile the 11 most commonly targeted cannabinoids in commercially available products. The UHPLC conditions achieve excellent chromatography including separation of Δ8-THC and Δ9-THC to a detection limit of 1.0 ng/mL (1E-7 wt %) with linear regression coefficients of 0.995 or better. These UHPLC conditions are translatable to any LC system making it platform agnostic which offers application flexibility ranging from panel specific, targeted applications for QA/QC through high-end discovery based methods for research and development purposes.

Keywords: Consumer Products, Liquid Chromatography
Application Code: Quality/QA/QC
Methodology Code: Liquid Chromatography
Analysis of cannabinoids is critical to the cannabis industry for product labeling and because product value is often based primarily on potency values. Although no standardized method exists, liquid chromatography with ultraviolet detection, LC-UV, has become the most popular technique. There is concern potency values are not consistent among labs even when the same technique is used. Methods typically consist of a simple extraction and dilution before LC-UV analysis. Sample cleanup is typically not performed except in the case of edible products. Thus, coextracted compounds remaining in the sample can potentially interfere with cannabinoid quantitation. Evidence exist indicating wavelength selection is critical because coextracted compounds, like terpenes, can coelute with cannabinoids and produce significant signal at common wavelengths used for cannabinoid detection causing inaccurate potency values. A simple isocratic chromatographic method was employed that allows resolution of isobaric cannabinoids. This work takes advantage of the selectivity of liquid chromatography- tandem mass spectrometry, LC-MS/MS, to quantify cannabinoids in a variety of cannabis matrices. Potential interferences were determined by comparing and identifying differences between LC-MS/MS data with LC-UV data. Suspected interferences identification was performed by scan GC-MS via library matching. Retention times and UV responses at different wavelengths of authentic terpene standards were used to determine if typical cannabis terpenes coelute with target cannabinoids. Comparative LC-UV and LC-MS/MS potency values of various matrices (flower, concentrates and edibles) will be presented. Interferences are determined and identified when possible. Terpenes were characterized using LC-UV to better understand their potential signal interference. Recommendations to ensure accurate quantitative data of cannabinoids will be discussed.

Keywords: Chromatography, Liquid Chromatography/Mass Spectroscopy, Mass Spectrometry, UV-VIS Absorbance
Application Code: Other
Methodology Code: Liquid Chromatography
In this talk, I will present our research on the use of single-molecule and single-particle fluorescence microscopy to study dynamic processes at the electrochemical interface. In one example, we studied the dynamic collision and dissolution of single silver nanoparticles at the surface of platinum nanoparticle electrode. In another example, we used single-molecule fluorescence microscopy to study dynamic generation and diffusion of individual nanobubbles. These results demonstrate the power of fluorescence microscopy in studying and understanding the electrochemical interface.

Keywords: Electrode Surfaces, Fluorescence, Imaging, Spectroelectrochemistry

Application Code: Nanotechnology
Methodology Code: Electrochemistry
Emerging Frontiers in Nanoscale Optical Imaging

Electrochemical Zero-Mode Waveguide Arrays for Imaging Single Reaction Events

Single electron transfer events in both immobilized and freely diffusing redox-active molecules can be imaged with facility using electrochemical zero-mode waveguide (E-ZMW) arrays. These bimodal nanoelectrochemical-nanophotonic nanopore arrays present high density recessed dual-ring electrode nanopores. Thus, they provide a link between single electron-transfer events and light emission in fluorogenic redox reactions, such as that of flavin mononucleotide, FMN. The isoalloxazine chromophore of FMN is fluorescent in the oxidized state, while the reduced state, FMNH2, is not - thus permitting the redox state of single FMN molecules to be followed by observing their fluorescence behavior. The bimodal optical-electrochemical functionality of the E-ZMW makes it possible to perform single molecule spectroelectrochemical measurements under redox cycling conditions – both when the upper electrode is potential-controlled and using self-induced redox cycling. Single molecule cycling is evidenced by single molecule electrochemical-spectroscopic cross-correlation measurements, which are used to study single molecule fluctuations in nanopore occupancy. The ability to combine electrochemical and spectroscopic measurements at the single molecule level is a new tool for the characterization of reaction dynamics.

Keywords: Electrochemistry, Fluorescence

Application Code: Nanotechnology

Methodology Code: Electrochemistry
Understanding nanoscale protein dynamics at interfaces is crucial for topics ranging from disease inception to drug delivery to separations science. Recent efforts by our group and others have shown the promise of applying single molecule methods to link mechanistic detail about protein adsorptions to macroscale observables. However, although the best super-resolution methods achieve high spatial resolution, the temporal resolution remains low. We report Super Temporal-Resolved Microscopy (STReM) to improve the temporal resolution of 2D super-resolution microscopy by a factor of 20 compared to the traditional camera-limited frame rate. This is achieved by rotating a phase mask in the Fourier plane during data acquisition and then recovering the temporal information by fitting the PSF orientations. The feasibility of this technique is verified with both simulated and experimental 2D adsorption/desorption and 2D emitter transport. When STReM is applied to measure protein adsorption at a glass surface, previously unseen dynamics are revealed.

**Keywords:** Fluorescence, Separation Sciences, Single Molecule, Spectroscopy

**Application Code:** Pharmaceutical

**Methodology Code:** Fluorescence/Luminescence
We describe new methods for expansion microscopy super-resolution imaging in which fluorophores labeling fixed specimens are linked to swellable polymers that enable nanoscale imaging with conventional microscopes and conventional fluorescent probes. We apply these methods to a variety of biological systems, including cultured cells, tissue sections, microbes, and small organisms, revealing previously unknown details.
Emerging Frontiers in Nanoscale Optical Imaging

Single Objective Light-Sheet Microscopy for Multi-Color Whole-Cell 3D Super-Resolution

Single molecule Super-Resolution in 3D throughout a whole cell is made more difficult by high background fluorescence that can degrade detection and localization precision. We have developed a technique, termed Single Objective Light-Sheet Microscopy (SO-LSM) that uses a single objective, inverted epi-fluorescence microscope along with a reflective surface to generate illumination only in the image focal plane. We use a reflective surface, placed at an angle of 45° on a cover slip to reflect the beam. This surface forms the side wall of a microfluidic channel incorporated into a microfluidic device. A light-sheet is generated through the objective and reflected such that it illuminates the in-focus plane of the cell. The light-sheet is scanned through the cell for whole-cell imaging while PSF engineering in the emission path enables the 3D localization of individual emitters, creating a whole-cell 3D super-resolution image. SO-LSM provides improved localization accuracy due to background reduction and reduced photobleaching of un-imaged, out-of-focus fluorophores. The microfluidics device provides a closed environment that allows for fast and automated buffer exchange, which is utilized to implement multi-structure imaging using a sequential approach.

Keywords: Biotechnology, Imaging, Microscopy, Single Molecule
Application Code: Biomedical
Methodology Code: Microscopy
Microphysiological systems (best recognized as ‘organ chips’) are a rapidly developing in vitro technology that is providing unique opportunities to model the complexity of in vivo biology. The growing debate about the role of animal studies in a challenging rate of drug development attrition for both efficacy and safety has prompted excitement for the potential for greater clinical relevance of human-sourced microphysiological systems over traditional animal models. Attaining the full promise and impact of this innovation will take a very deliberate process of evolution. This presentation will outline a framework used at AstraZeneca to select the context of use for microphysiological systems and exemplify the type of data that is possible to achieve from such systems in drug development.
Human-on-a-Chip Systems for Toxicology and Drug Discovery

Organs-on-Chips: A Platform for Advancing Efficacy and Safety Testing in Drug Discovery

Human “Organs-on-Chips” are microengineered systems that recapitulate the tissue microenvironment. Each Organ-Chip is composed of a clear flexible polymer about the size of a AA battery that contains hollow channels lined by living cells. The Chips are cultured under continuously flow within engineered 3D microenvironments that go beyond conventional 3D in vitro models by recapitulating in vivo intercellular interactions, spatiotemporal gradients, vascular perfusion, and mechanical microenvironments. Integrating cells within Organs-on-Chips, enables the study of normal physiology and pathophysiology in an organ-specific context. One of the novel and distinctive features is the level of cellular and molecular resolution that enables key insights into the mechanisms of action of drugs in a human relevant context. In this presentation will highlight studies with various academic, clinical, and industry collaborators to demonstrate the utility of the system as a more predictive human-relevant alternative for efficacy and safety testing of new chemical entities in humans. The data demonstrates the ability to model normal and disease physiology in organs such as lung, intestine, and liver. One such example will include the application of the Liver-Chip. We have demonstrated that the Liver-Chip can support improved viability and hepatic function over conventional hepatocyte culture systems. During long-term culture we demonstrated the Liver-Chip maintains stable physiologic levels of key hepatic functions such as albumin secretion, urea secretion, and expression of CYP450s drug metabolizing enzymes. Using the Liver-Chip we were able to recapitulate toxicity findings in the clinic that were not previously observed in other in vitro systems or animal models. Implementation of Organ-on-Chips within the pharmaceutical industry aims to improve the probability of success of drugs by generating models that are more human and disease relevant.

Keywords: Biomedical, Drug Discovery, Lab-on-a-Chip/Microfluidics, Toxicology
Application Code: Drug Discovery
Methodology Code: Microfluidics/Lab-on-a-Chip
The renal proximal tubule is a primary site of acute kidney injury given its ability to concentrate drugs/xenobiotics, via facilitated transport, to levels higher observed than in the circulating plasma. Because conventional 2D cultures of proximal tubule epithelial cells lack many key properties required for predictive toxicity testing (e.g. polarity, transporter expression/activity, extended viability, etc.) we have developed a three dimensional microphysiological system (MPS) of the human proximal tubule. Previously published studies have shown the MPS exhibits sensitivity to acute kidney injury (AKI) in response to the heavy metal pollutant cadmium. A more recent application of the MPS has involved evaluating polymyxin B and structural variants. Polymyxin antibiotics are a last-line therapy for treating drug-resistant bacteria; their use is limited due to the high frequency (~30%) of AKI. We are using the kidney MPS to model the acute (48 h) effects of polymyxin B treatment (50 µM) in the MPS as well as to ascertain the safety of structural analogs designed to lower toxicity (due to increased renal clearance) while retaining anti-microbial efficacy. We have shown that the MPS is sensitive to polymyxin B-induced AKI, exhibiting significant induction of urinary biomarkers (kidney injury molecule-1 and multiple microRNAs), cell-associated biomarker (heme-oxygenase-1) induction, and transcriptional responses (induced cholesterol biosynthesis pathway and ROS stress response). Analysis of the pathways induced by polymyxin B provide insight into the mechanisms of AKI as well as giving information on potential interventions to mitigate toxicity. In addition, the robustness of our MPS was demonstrated by the low variability in global gene expression when comparing multiple MPS from the same donor, while also providing information on donor to donor variability.

Keywords: Biotechnology, Clinical/Toxicology, Pharmaceutical, Toxicology
Application Code: Clinical/Toxicology
Methodology Code: Microfluidics/Lab-on-a-Chip
Human-on-a-Chip Systems for Toxicology and Drug Discovery

The art of drug discovery would benefit greatly from better pre-clinical screening technologies to reduce the attrition rate during clinical trials as well as to begin to pre-select specific genetic sub-populations for optimal drug efficacy with limited off target toxicity. A promising technology to reduce the cost and time of this process are human-on-a-chip or body-on-a-chip systems. This can be at either the single organ level, or more importantly, advanced systems where multiple organ mimics are integrated in a serum-free defined medium under flow to allow organ to organ communication and interactions for mechanistic investigations for not only safety but efficacy as well. The idea is to integrate BioMEMs devices and surface modifications with protein and cellular components, for initiating and maintaining self-assembly and growth into biologically, mechanically and electronically interactive functional multi-component systems. A functional neuromuscular junction model composed of human stem cell derived motoneurons and muscle has been used to produce dose response curves for synaptic cleft toxins. Cardiac modules have been developed that allow independent evaluation of electrical conduction and force generation in cardiac muscle for mechanistic studies for both human iPSC and embryonic stem cells. These have also been combined with liver systems in the same platform to include metabolic effects that have been demonstrated with 4 different drug/metabolic pairs. Recently a 4-organ system with recirculating medium was demonstrated for 28 days and exhibited toxicity with 5 drugs that reproduced in vivo results. Overall there appears to be outstanding potential to apply these multi-organ human-on-a-chip systems to understand mechanistic toxicology and efficacy. Detailed results with the above system will be presented as well as results from collaboration with industry partners.

Keywords: Drug Discovery, Integrated Sensor Systems, Lab-on-a-Chip/Microfluidics, Microelectrode
Application Code: Pharmaceutical
Methodology Code: Microfluidics/Lab-on-a-Chip
Drug induced liver injury (DILI) is still a major source of clinical attrition and post market withdrawal of drugs. This is due to the many different etiologies of DILI, the relatively low incidence rate, poor understanding of the drivers for patient susceptibility, and a general insensitivity of preclinical animal models to detect most human hepatotoxicants. Extensive efforts are being made to evaluate and to employ more physiologically-relevant in vitro models to assess risk for hepatotoxicity in drug discovery. Accordingly, new commercially available microphysiological hepatic models have emerged that present several advantages over traditional in vitro hepatocyte models, including but not limited to long-term treatment durations, sustained and stable metabolic activity, and added complexity (e.g. inclusion of non-parenchymal cells). This presentation will focus on characterization, qualification, and implementation of spheroid human liver microtissues (hLiMTs) comprised of both primary hepatocytes and non-parenchymal cells (e.g. Kupffer cells) to support drug-discovery. In particular, we assessed the predictive value of long-term (14-day treatment) cytotoxicity in (hLiMTs) in comparison to acute (48h) cytotoxicity in plated primary human hepatocytes (PHH) alone for 110 drugs (60% DILI positive and 40% DILI negative). Regardless of comparing cytotoxicity IC50 values or exposure-corrected margin of safety values, hLiMT demonstrated increased sensitivity in identifying known hepatotoxicants than PHH, while specificity was consistent across both assays. In addition, hLiMT out performed PHH in correctly classifying hepatotoxicants from within a pharmacological class. Description of how this assay is used to assess hepatotoxicity risk assessment in drug discovery as well as case studies on how this assay was employed to explore mechanisms of hepatotoxicity for drugs associated with preclinical and/or clinical hepatotoxicity will be presented.

Keywords: Drug Discovery, Drugs, Pharmaceutical, Toxicology
Application Code: Safety
Methodology Code: Microfluidics/Lab-on-a-Chip
Abstract Text
Wearable sensors have garnered considerable recent interest owing to their tremendous promise for a wide range of healthcare, military, sport and wellness applications [1]. While early studies have been devoted for the monitoring of vital signs, recent efforts have focused on the non-invasive monitoring of chemical markers (e.g., metabolites, electrolytes). Such wearable sensing platforms provide new avenues to continuously monitor individuals and can thus tender crucial information regarding a wearer’s health and performance in real time. The growing sophistication of these new wearable devices is leading to the realization of new ‘lab-on-a-skin’ and ‘Lab-in-the-Mouth’ analytical platforms.

This presentation will discuss our recent developments of wearable chemical sensors integrated directly onto both textile materials and on the epidermis as well onto a mouthguard for various non-invasive monitoring [2]. The preparation and characterization of such textile-based and skin-worn electrochemical sensors requires resiliency against mechanical strains in connection to new stretchable and self-healing materials. Mechanical stress studies indicate that tattoo and textile-based printed electrochemical sensors survive large deformations. Portable flexible electronic boards allow transmission of the results wirelessly to the user’s smartphone. Technical challenges and prospects for using textile- and tattoo-based electrochemical sensors for monitoring the wearer’s health, fitness, or surrounding environment will be discussed, along with several demonstrations and prospects for future healthcare and sport applications.

Keywords: Sensors
Application Code: Bioanalytical
Methodology Code: Integrated Sensor Systems
NextGen nucleic acid sequencing has proven to be transformative for understanding the genetics of organisms. Beyond this, though, it should be appreciated that NextGen sequencing is perhaps one of the most remarkable analytical tools ever invented. Building off of the extraordinary ability of PCR to amplify and thereby provide readouts at the single molecule level, NextGen sequencing provides parallelism of information acquisition for literally millions to billions of molecules at the same time. Unfortunately, these molecules are for the most part DNA and RNA. What if there were ways to transmogrify other types of molecules or analytes into DNA, so that chemometric methods could be used to analyze virtually any analyte? The field of so-called NextGen chemometrics explores this by providing biosensors that lead from analytes to binding or catalysis to sequence information, ultimately providing detailed molecular readouts for virtually any analytical application.

Keywords: Bioanalytical, Bioinformatics, Biosensors, Nucleic Acids
For four decades, I have enjoyed working with interdisciplinary teams to solve problems in unconventional ways. In the ‘70’s I spearheaded a team that reported the first method for detecting circulating tumor cells. Using “new” flow cytometer technology, we detected small clonal populations of B lymphocytes from solid tumors based on identical surface concentrations of immunoglobulins with kappa or lambda light chains. That approach is still used today in some oncology clinics for diagnosis and treatment monitoring of lymphoma patients. In the ‘80’s, at the Naval Research Laboratory, we invented the displacement immunoassay for continuous monitoring of small molecules based on an approach that defied the textbook description of antibody binding kinetics—eventually, we even figured out why it worked and licensed the technology for detection of drugs of abuse, explosives, and environmental pollutants. Around 1990, my team solved the problem of getting excitation light into the evanescent wave over centimeters of optical fiber and collecting the fluorescent signal with high efficiency—making fluorescence-based optical biosensors practical for detection of biological warfare agents. Later in the ‘90s, we successfully deployed a 10-pound, automated system on a small drone that was capable of collecting target from the air into water, immunoanalysis, and remote data transmission. In the early 2000’s, we used techniques developed for microfluidic mixing to position unmixed streams in a pre-planned configuration, polymerizing fibers with novel shapes. In this decade, we incorporated living cells into those fibers to create microcapillaries capable of sprouting, anastomosis, and interactions with cells in surrounding 3D tissues—both on chip and in vivo. Working across science and engineering disciplines never fails to generate a plethora of new ideas!
The “Internet of Things” (or IoT) is a phrase that describes how more and more machines and devices are connecting and directly communicating with each other – whether it is your car sending information to your insurance company’s server, or your home thermostat sending messages to your phone... or your -80 freezer sending you a text (and also telling your LIMS system) that your irreplaceable samples are about to thaw out because the door was not closed properly.

The IoT is transforming virtually every workplace, and biotech is no exception. IoT technologies specifically applied to the world of biology are bringing the rigor and automation that prevail in manufacturing to earlier stages of scientific product research and discovery, from pharma to synthetic biology. By automatically gathering new data streams for scientists – filling data “gaps” in their work – and automating the execution of protocols to eliminate human variability, the implications include faster time to results, faster time to market, and better understanding of scientific processes.

Simply put, harnessing the technologies that have provided the building blocks for the IoT and applying them to scientific processes has widespread benefits. It helps teams identify unknown factors that may be contributing to outcomes – essentially the proverbial “needle in the haystack” – and accelerates discovery. Automation will also transform the role of scientists and technicians - technology will streamline processes so employees can be more efficient and strategic. Teams no longer need to use staff to manually record, enter and model data. Nor do teams need to be in the lab 24x7 to monitor their experiments. Instead staff can use their time for more value-added work to further optimize team performance.

This talk will explore how IoT enhances automation efforts and fundamentally reshapes science-based work environments and the implications for people in all roles – from research to operations management.

Abstract Text

Keywords:

Automation, Lab Management, Laboratory Informatics, Process Monitoring

Application Code:

Laboratory Management

Methodology Code:

Laboratory Informatics
Revolutionary science often requires thinking outside of the box. However, stepping outside of the box can be fraught with many hazards, not the least of which is the loss of objectivity.

Scientists are often not very good judges of the scientific process. The best intentions can be subverted by self-deception. Even eminent scientists have had their careers tarnished or ruined by misinterpreting unremarkable events and convincing themselves that they have made a great discovery.

Error is a normal part of science, and many revolutionary discoveries turn out to be wrong. Uncovering flaws in observations or reasoning is part of what scientists do, by replicating measurements and designing control experiments.

"Pathological science" is the term coined by Irving Langmuir in 1953. Other terms for this phenomenon include: pseudoscience; voodoo science; junk science; weird science; etc. However, pathological science should be distinguished from hoaxes and fraud -- which are intentionally meant to deceive.

This presentation will look at the various causes of pathological science and examine some of the interesting examples, such as Martian canals, N-rays, 21-gram soul, extrasensory perception, polywater and cold fusion.

Self-deception can affect anyone -- scientists are only human -- even some of the best minds have been fooled. Critical thinking must be used to avoid the pitfalls of pathological science.

## Keywords
Data Analysis, Education, Scientific Data Management

## Application Code
General Interest

## Methodology Code
Education/Teaching
MALDI Imaging Mass Spectrometry (IMS) 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 of this nature are produced in specific m/z (mass-to-charge) values, or ranges of values. Each imaged specimen gives rise to many hundreds of specific molecular images from a single raster of the tissue. In a complementary approach where only discrete areas within the tissue are of interest, we have developed a histology-directed approach that integrates mass spectrometry and microscopy.

We have employed IMS in studies of a variety of biologically and medically relevant research projects, several of which will be presented including studies in diabetic nephropathy involving both proteins and lipids and the differentiation of benign skin lesions from melanomas. In addition, IMS has been applied to drug targeting and metabolic studies both in specific organs and also in intact whole animal sections following drug administration.

This presentation describes recent technological advances both in sample preparation and instrumental performance to achieve images at high spatial resolution (1-10 microns) and at high speeds so that a typical sample tissue once prepared can be imaged in minutes. Instrumentation used in these studies includes both MALDI FTICR MS and MALDI TOF mass spectrometers. Applications utilize MS/MS, ultra-high mass resolution, and ion accumulation devices for IMS studies. Finally, new biocomputational approaches will be discussed that deals with the high data dimensionality of IMS and our implementation of ‘image fusion’ in terms of predictive integration of MS images with microscopy and other imaging modalities.

Keywords: Biomedical, Mass Spectrometry, Microscopy, Molecular Spectroscopy
Application Code: Biomedical
Methodology Code: Mass Spectrometry
A New Method for Detection of Region- and Enzyme-Specific Bioconversion of Neuropeptides by Combining In Situ Enzyme Histochemistry and MALDI Imaging Mass Spectrometry

Region-specific expression of proteolytic enzymes controls the biological activity of endogenous neuropeptides and has recently been targeted for the development of novel drugs, for example in neuropathic pain, depression, and for L-DOPA-induced dyskinesia in Parkinson’s disease. Rapid and sensitive analytical methods to profile modulators of enzymatic activity are important for finding effective inhibitors with high therapeutic value.

Here we combined in situ histochemistry with MALDI imaging mass spectrometry and demonstrate that this is a highly sensitive method for analysis of brain-area specific neuropeptide conversion of synthetic neuropeptides, and for selection of peptidase inhibitors that differentially target conversion enzymes at specific anatomical sites. Dynorphin B (DynB) was used as model neuropeptide and effects of peptidase inhibitors applied to brain tissue sections were analyzed. Synthetic DynB (2pmol) was found to be converted to both N-terminal and C-terminal fragments. Several specific and non-specific inhibitors were tested, some of which completely blocked conversion of fragments at 20 attomols on target. We also show dose-dependent inhibition of bioconversion and strain-specific differences in the bioconversion of DynB.

Bioconversion of synthetic DynB was region-specific, producing DynB(1-7) in the cortex and DynB(2-13) in the striatum. Both phosphoramidon (inhibitor of neprilysin) and opiorphin (inhibitor of neprilysin and aminopeptidase N) blocked cortical bioconversion to DynB(1-7), whereas only opiorphin blocked striatal bioconversion to DynB(2-13).

This new unbiased method ISH-MALDI imaging MS will be a very effective tool in the development of novel pharmaceuticals targeting enzyme activity, as it requires no labeling and detects both substrates and metabolites in one single experiment. It can reveal multiple cleavage sites and indicate if several enzymes are involved in bioconversion.

Keywords: Clinical/Toxicology, Imaging, Mass Spectrometry, Method Development
Application Code: Clinical/Toxicology
Methodology Code: Mass Spectrometry
Mass spectrometry provides multiple options for the direct characterization of tissue to support surgical decision-making, and provides significant insight in the development of drugs targeting tumors of the central nervous system (CNS). Using an array of mass spectrometry (MS) applications, we rapidly analyze specific tumor markers ranging from small metabolites to proteins from surgical tissue for rapid diagnosis and surgical guidance. Using similar clinical protocols, we visualize drug and metabolites penetration in brain tumor tissue and correlate with tumor heterogeneity and response to support drug development.

**Abstract Text**

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

**Application Code:** Biomedical

**Methodology Code:** Mass Spectrometry
In recent years, imaging mass spectrometry (IMS) has become the leading technique for the analysis of biomolecules directly from thin tissue sections while retaining the biomolecules’ anatomical distributions. In recent years, IMS greatly improved in spatial resolution due to the advancement in both sample preparations and instruments to the point of reaching cellular level capabilities (\(10 \, \mu m\)). Indeed, the need to develop tools for imaging molecular features by IMS on a single tissue section becomes a necessity to observe and characterize smaller tissue structures. In this presentation, new developments in MALDI-TOF IMS from our laboratory will be presented and their potentials for the detection and characterization of molecular changes in neurodegenerative or related diseases will be exemplified and discussed. In particular, a method for enhanced detection of amyloid beta peptides has been developed and applied to detect their presence in early stages of Alzheimer’s disease. A second method has been developed to specifically enhance the detection of gangliosides in brain tissue. This approach has been applied to monitor the enhanced presence of such molecules in neuroblastoma.

**Keywords:** Imaging, Lipids, Mass Spectrometry, Protein

**Application Code:** Neurochemistry

**Methodology Code:** Mass Spectrometry
In the postgenomic era, one expects the suite of chemical players in a brain region to be known and their functions uncovered; however, many important compounds are not well characterized, especially analytes found in uncommon cells. A suite of matrix-assisted laser desorption/ionization (MALDI) mass spectrometry-based approaches are described that allow us to assay individual cells and small brain regions; these approaches include mass spectrometry imaging and single cell mass spectrometry based profiling. We are able to characterize lipids, fatty acids, metabolites, peptides and many other analytes directly from the tissue slides. Depending on the analytes to detect, a variety of approaches are used to enhance the signals from specific analyte classes, including using specialized matrices, nanoparticles, and on-tissue derivatization. With appropriate standards, relative and even absolute amounts of analytes can be determined. For single cell profiling, we randomly disperse thousands of cells onto a microscope slide, the individual X-Y cell coordinates are determined by fluorescence microscopy before being coated with a chemical matrix. MS profiling is then performed at the location of each cell. Since cells stay adhered to the slide and the MALDI MS measurement only consumes a fraction of the sample, this experimental approach is flexible, allowing integration of other techniques either pre- or post-analysis. We have re-assayed selected cells with another mass analyzer, immunohistochemistry, transcriptomics, capillary electrophoresis-mass spectrometry and other approaches. The results of our work provide an unprecedented glimpse into the chemical and cellular heterogeneity of the brain at the single cell level. MALDI MS allows us to uncover the complex chemical mosaic of the brain and pinpoint key cellular players in physiological and pathological processes.

**Keywords:** Imaging, Mass Spectrometry, Metabolomics, Metabonomics

**Application Code:** Neurochemistry

**Methodology Code:** Mass Spectrometry
Exosomes are an emerging new biomarker for cancer management. Exosomes are nanoscale vesicles (50 – 200 nm in diameter) actively secreted by cancer cells. These extracellular vesicles carry molecular constituents of their originating cells, and can thus serve as cellular surrogates that can be repeatedly and conveniently obtained with minimal complications. We have been developing new biosensors to streamline clinical exosome analyses. These systems include acoustic-wave based microfluidics for exosome isolation and nanotechnology-inspired sensors exosome molecular screening. The ensuing clinical studies with patient samples (glioblastoma multiforme and ovarian cancer) confirmed the potential of using exosome analysis to monitor tumor progression and treatment responses. Further clinical investigations are underway to rigorously evaluate the clinical utility of the developed systems.

**Keywords:** Bioanalytical, Biomedical, Biosensors

**Application Code:** Biomedical

**Methodology Code:** Sensors
To put disease-related biomarkers to work in the clinic, new high-performance technologies are needed to enable rapid and sensitive analysis of clinical specimens. Electrochemical methods providing low cost and direct biomarker read-out have attracted a great deal of attention for this application, but have, to date, failed to provide clinically-relevant sensitivity. We exploit controlled nanostructuring of electrode surfaces to promote surface accessibility and enhance capture rate and efficiency to solve this long-standing problem, and showed that the nanoscale morphologies of electrode surfaces control their sensitivities. Recently, we have developed assays that are able to detect nucleic acids, proteins and small molecules, with universally high sensitivity levels. This presentation will highlight how electrodeposited metals can be used to create high-performance sensors with applications in infectious disease diagnosis, cancer management, and transplantation medicine.

**Keywords:** Bioanalytical, Biomedical, Biosensors, Electrode Surfaces

**Application Code:** Biomedical

**Methodology Code:** Electrochemistry
Biological systems use complex ‘information processing cores’ composed of molecular networks to coordinate their external environment and internal states. An example of this is the acquired, or adaptive, immune system (AIS), which is composed of both humoral and cell-mediated components. Here we report the step-by-step construction of a prototype mimic of the AIS which we call Adaptive Immune Response Simulator (AIRS). DNA and enzymes are used as simple artificial analogues of the components of the AIS to create a system which responds to specific molecular stimuli in vitro. We show that this network of reactions can function in a manner which is superficially similar to the most basic responses of the vertebrate acquired immune system, including reaction sequences that mimic both humoral and cellular responses. As such, AIRS provides guidelines for the design and engineering of artificial reaction networks and molecular devices. We will also discuss other DNA nanostructures for molecular medicine.

Keywords: Bioanalytical, Biosensors, Nanotechnology, Nucleic Acids
Application Code: Bioanalytical
Methodology Code: Sensors
Integration of dual-barrel membrane patch-ion channel probes (MP-ICPs) to scanning ion conductance microscopy (SICM) holds promise of providing a revolutionized approach of spatially-resolved chemical sensing. However, to fully exploit the MP-ICP platforms for concurrent topography and chemical sensing, a comprehensive characterization of the analytical performance of this newly-developed platform is needed. A series of experiments were performed to further our understanding of the system and to answer some fundamental questions, in preparation for future developments of this approach. First, we constructed MP-ICPs that contained different types of ion channels including TRPV1 channels and BK channels to establish the generalizability of the methods. Next, we proved the capability of the MP-ICP platforms in single ion channel activity measurements, and demonstrated that the channel behaviors can be faithfully obtained with this approach. In addition, we studied the interplay between the SICM barrel and the ICP barrel. An interesting phenomenon that’s related to the charge of the ligand molecules was discovered, for ion channels gated by uncharged ligands, channel activity at the ICP barrel is unaffected by the SICM barrel potential; whereas for ion channels that are gated by charged ligands, enhanced channel activity can be obtained by biasing the SICM barrel at potentials with opposite polarity to the charge of the ligand molecules. Finally, a proof-of-principle experiment was performed and site-specific molecular flux sensing was demonstrated at single-ion-channel level.

Keywords: Bioanalytical, Electrochemistry
Application Code: Bioanalytical
Methodology Code: Electrochemistry
Quantitative analysis of circulating biomarkers in cancer is critical for developing liquid-biopsy based methods for non-invasive diagnosis and monitoring of therapy response. However, in many cases circulating biomarkers (e.g., ctDNA, circulating tumor cells, and tumor-derived exosomes) are present at very low concentration levels, especially at the early stages of disease. In comparison with nucleic acids that can be amplified, quantitative detection of low-level proteins and extracellular vesicles remains very challenging. Here we will present new microfluidic bioanalytical platforms functionalized with nanomaterials, such as graphene nanomaterials, for ultrasensitive analysis of circulating biomarkers with broad dynamic range. These nano-/micro hybrid technologies substantially enhance the analytical metrics while reducing the sample demand and analysis time. As a proof of concept for clinical applications, we have adapted these microfluidic platforms to quantitatively measure protein biomarkers and tumor-derived exosomes in the circulation as a means for liquid biopsy based non-invasive diagnosis of cancer diagnostics. Overall, these nanomaterial-inspired microfluidic systems would provide enabling bioanalytical technologies to promote quantitative measurement of complex biological systems and clinical disease diagnosis.

Keywords: Biomedical, Immunoassay, Lab-on-a-Chip/Microfluidics, Nanotechnology
Application Code: Biomedical
Methodology Code: Microfluidics/Lab-on-a-Chip
Within each living organism proteins are at work, carrying out activities which impact every aspect of cellular function from synthesis to cell death. The next generation of medicines will rely heavily upon our ability to quickly assess the structures and stabilities of such complex macromolecular machines, as well as the influence of large libraries of conformationally-selective small molecule binders and protein-based biotherapeutics. Such endeavors are nearly insurmountable with current tools. In this presentation, I will discuss recent developments surrounding the activation of gas-phase protein complex ions aimed at bridging this gap in basic technology. One such development is collision induced unfolding (CIU), which uses ion mobility-mass spectrometry (IM-MS) to measure the stability and unfolding pathways of gas-phase proteins, without the need for covalent labels or tagging, and consuming 10-100 times less sample than almost any other label-free technology. In parallel with this approach, my lab are pursuing chemical modification strategies aimed at the improved liberation of sequence informative peptide fragments from intact protein complex precursor ions during collision induced dissociation (CID), enabling the assessment of protein quaternary structure and sequence simultaneously. Recent developments in understanding the mechanisms of protein CIU and CID, the ability of these tools to differentiate therapeutic antibodies and enable the discovery of conformationally-selective inhibitors, will be discussed.

Keywords: Biopharmaceutical, Electrospray, Mass Spectrometry, Tandem Mass Spec
Application Code: Pharmaceutical
Methodology Code: Mass Spectrometry
The field of native mass spectrometry is rapidly growing and evolving due to a variety of factors. Largely, the expanded availability of instrumentation that is capable of such measurements, combined with the demonstration of value of direct measurement of protein-protein and protein-ligand interactions by a select few innovators and early adopters is driving a significant increase of effort in this area. However, much of the focus in this area is on the high fidelity elucidation of protein complex structure. In contrast to these targeted measurements, our contribution has been the development of a platform that operates in the middle ground between high throughput denaturing proteomics and low throughput structural mass spectrometry. Herein, we will describe a growing dataset that includes more than 100 multiproteoform complexes and discuss what we have learned from their characterization. Our observations raise provocative questions about the prevalence of certain post-translational modifications, reveal novel metal binding events, and show examples of enzyme-substrate interactions being directly observed, all from a simple crude cell lysate.

**Keywords:** Bioanalytical, Mass Spectrometry, Protein, Proteomics

**Application Code:** Bioanalytical

**Methodology Code:** Mass Spectrometry
Membrane proteins play critical biochemical roles, but they remain challenging analytical targets. Native mass spectrometry holds great potential to provide a more complete picture of membrane protein structure, function, and interactions. However, conventional native mass spectrometry of membrane proteins has relied on detergent micelles, which are not physiological. Nanodiscs provide a more natural lipid bilayer environment for studying membrane proteins and protein-lipid interactions, but they present unique challenges in sample preparation and data analysis. We will present advances in native MS analysis of Nanodiscs, including engineering the membrane scaffold protein belt for MS, charge reduction to improve the spectra, and improved methods for membrane protein analysis. These advances open new doors for studying membrane proteins in nanoscale lipid bilayers by mass spectrometry.

Keywords: Bioanalytical, Biotechnology, Mass Spectrometry
Application Code: Bioanalytical
Methodology Code: Mass Spectrometry
Protein footprinting coupled with mass spectrometry has emerged in recent years as a valuable tool to study protein structure. One of these footprinting methods, hydroxyl radical footprinting (HRF), utilizes hydroxyl radicals to oxidatively modify the side chains of solvent accessible amino acids. The solvent accessibility of amino acids change upon ligand binding and protein conformational change and these changes are reflected in the oxidative modification pattern. There are multiple methods to generate hydroxyl radicals including Fenton chemistry and radiolysis of water. One such method, fast photochemical oxidation of proteins (FPOP), utilizes an excimer laser for photolysis of hydrogen peroxide to generate hydroxyl radicals. FPOP has been successfully used to characterize protein-protein and protein-ligand interaction sites as well as regions of conformational change. We have extended the use of FPOP for analysis of proteins inside cells. This method, entitled in-cell FPOP (IC-FPOP), makes it possible to study proteins in their native cellular environment. The method probes solvent accessibility similar to in vitro FPOP demonstrating its potential for studying protein structure within the cell. We have used the method to study the GCaMP2 chimeric protein. Preliminary results indicate IC-FPOP can detect regions of conformational change in GCaMP2 upon calcium binding.
Membrane proteins are essential to mediate the traffic of solutes in and out of the cell, and in translating extracellular stimuli into function. Membrane proteins can be challenging to structurally characterize with traditional techniques, due to their low expression yields and difficulty in producing diffraction quality crystals. Mass spectrometry is emerging as a powerful structural biology tool, enabling analysis of intact soluble and membrane protein complexes, their proteoforms, and well as membrane protein-lipid complexes. Native MS is especially powerful when coupled with dissociation techniques such as collision induced dissociation, or surface induced dissociation. SID is a promising technique for such studies as it dissociates a protein complex into its structurally informative subunits without perturbing protein structure. Here we report on the application of ion mobility-mass spectrometry coupled with surface induced dissociation (SID) to study membrane protein complex interactions as well as protein:lipid interactions. In addition, we will use high resolution MS and SID to further characterise the proteins and their interactions. In particular, we will focus on the study of the membrane protein Aqp0. AQP0 is the most abundant membrane protein expressed in the eye lens, making up >50% of the membrane protein content. Interestingly, mutations in this protein can cause congenital cataract. We have isolated Aqp0 directly from bovine lens and have studied the intact tetramer by native MS with SID. We observe both phosphorylated and nonphosphorylated protein and interestingly also observe endogenous lipid being retained on a portion of the protein, even after purification and buffer exchange.
The field of point-of-care (POC) diagnostics offers the tantalizing possibility of providing rapid diagnostic results in nonlaboratory settings. First, we provide an overview of significant technological and social trends, notably those concerning data connectivity, which have shifted the underlying landscape for how POC diagnostic devices will be designed, built, and delivered across different healthcare settings. We review important technical advances in fundamental diagnostic components and, increasingly, advances in fully integrated devices designed for specific clinical use cases. While few new classes of POC diagnostic devices have been introduced into the market, continued progress in microfluidics, combined with dramatic advances in connected devices, is bringing the prospects of fulfilling the lofty promises of POC diagnostics closer than ever to reality.

With the rise of connected consumer devices, entire sectors of the economy (including retail, transportation, housing, and freelancing services) have been unmistakably transformed. The potential reach of POC diagnostics into all sectors of healthcare, and increasingly into daily routines of individual patients and consumers, demands that technical advances take into consideration this broader transformation.

For healthcare providers, the landscape for medicine is poised for a dramatic shift. In any decentralized setting, a connected POC diagnostic device could be available to aid the diagnosis of disease and selection of treatments.

For consumers and patients, the impact could be even more dramatic. POC diagnostic and monitoring devices are expected to become ubiquitous, whether in an at-home setting, in a doctor’s office or hospital, or in low- and middle-income countries. We will review recent developments and trends in POC diagnostics, including work in assay chemistry and microfluidics, and describe how these developments have been evolving to align with broader trends.

Keywords: Biomedical
Application Code: Biomedical
Methodology Code: Microfluidics/Lab-on-a-Chip
The need for practical and low-cost cancer diagnostics is more pronounced in resource-limited settings with overburdened clinical infrastructures. This work describes a platform that leverages smartphone technology to rapidly detect and profile cancer cells labeled with marker-specific microbeads through generation of unique holographic signatures. Captured images are wirelessly transmitted to remote cloud servers for reconstruction and analyses. We developed a two-step labeling method that simplified reagent preparation by obviating primary antibody modification (i.e. removes need for costlier customized antibodies) and enabling use of generic microbeads for various markers. This presentation will highlight preclinical and clinical efforts advancing holographic technologies towards point-of-care ends across diverse geographies. Exploiting smartphone diagnostic terminals could empower resource-poor communities. Coupled with low costs and operator independent readouts, this holographic platform is poised to become a robust smartphone-based cancer screening tool. Future developments in wearable sensor technologies and deep learning could prove synergistic with such holographic strategies.
Metals are ubiquitous in the environment and have long been recognized to pose significant threat to human health. Manganese (Mn) is an essential element, yet neurotoxic in excess, capable of crossing the blood-brain barrier and accumulating in the brain. This talk will discuss recent developments in electrochemical metal determination for point-of-care assessment of Mn in clinical applications. While anodic stripping of Mn has been reported in literature, stripping analysis on microscale remains a critical challenge due to the strong negative potential of stripping peak or complexation with other metals. Necessity for low limits of detection, high reproducibility, and low (disposable) sensor costs present additional challenges. Our ultimate goal is to demonstrate rapid, point-of-care, multi-analyte assessment of Mn in a finger prick of blood.
### Abstract Text

Paper-based microfluidic devices offer many promising capabilities for use as point-of-care sensors. This talk will describe our recent work developing technologies for fabricating higher-resolution paper-based microfluidic devices, for stabilizing enzymes on paper, and for controlling capillary wicking in paper-based devices to enable more sophisticated, multi-step assays with minimal user input.

**Keywords:**  
Biosensors, Immunoassay, Paper/Pulp

**Application Code:**  
Bioanalytical

**Methodology Code:**  
Microfluidics/Lab-on-a-Chip
Implants such as plates, screws and pins that are used for broken bone repair are generally made of stainless steel and are permanent unless they are removed. Implants made of magnesium and its alloys that gradually dissolve after bone healing avoid the need for removal by a later surgery if complications arise. The rate of bioresorption of magnesium can be controlled by judicious choice of alloying metals and/or coatings. Magnesium bioresorbs by reacting with water to form soluble magnesium ion and hydrogen gas. The bioresorption of magnesium can be monitored by measuring the hydrogen evolved. Hydrogen measurement has proven to be especially useful for monitoring magnesium bioresorption during in vivo evaluation with test animals. Both electrochemical and visual hydrogen sensors that are sufficiently sensitive to measure the very low levels of hydrogen involved have been developed and demonstrated for this application. Monitoring hydrogen transdermally is easily done by placing the sensor on the skin above the implant where it responds to hydrogen permeating through the skin. Monitoring hydrogen transdermally by a simple, non-invasive procedure with a sensor provides an effective means for monitoring magnesium implants as they bioresorb. The procedure is easy and potentially useful for point-of-care applications such as patient follow-up checks in a doctor’s office.
Current fast changing and dynamic world provides vast readily available information and a variety of opportunities to people. While there are all kinds of tools and opportunities to help us, this world is also more demanding and distracted than ever for one wants to be successful.

To scientists in industry, company project is the “king”, but we are not only expected to deliver projects, we also need to be excellent in science and technology, publishing, working as individual contributors as well as in teams, and maybe also managing and developing people. While playing multiple roles at work, we of course want to spend quality time with family and friends.

As a scientist and a manager in industry, and a mother of two, I will share with you my experience, the growing of my mindset over the years and what I have learned from others. We will discuss grit, passion and deep work in the field of science, in addition to prioritizing, focusing and balancing. We will discuss managerial track, scientific track, and a life outside of work.

Keywords: Education, Teaching/Education
Application Code: General Interest
Methodology Code: Education/Teaching
# How to be Successful in Your Career

## Analytical Careers in Industry

The range of roles in which Analytical Chemists contribute to Research & Development have grown over the years. In many ways, Analytical Chemists are more than ever integral to the Research & Development enterprise. This talk will focus on sharing insights into roles in which Analytical Chemist play in Industry. For students who are interested in a career as an Analytical Chemist, you will gain insight on skills required to gain access to career opportunities. For seasoned professionals looking to transition into the Analytical Chemistry profession, you will gain insight on job market opportunities for Analytical Chemist. At the end of this presentation, attendees should have a better understand of the multifaceted opportunities for Analytical Chemist in Industry.

**Keywords:** Analysis, Chemical, Chromatography, Data Analysis

**Application Code:** Laboratory Management

**Methodology Code:** Education/Teaching
How to be Successful in Your Career

Have You Considered a Career in the Analytical Instrument Field?

Do you like the challenges in working in the leading edges of analytical chemistry? Do you enjoy working with a team of equally motivated scientists? Then, you may want to consider a career in the analytical instrumentation and methodology field. This talk will focus on the opportunities in a wide variety of jobs that are available in analytical-oriented instrumentation development and related sciences. With opportunities ranging from basic research and development to the business and management side of this industry, there is a vast variety of jobs that will satisfy every type of personality and interest. Before retirement, I worked in this environment for 42 years in R/D, applications, technical support, instrument and column development in LC, GC and sample prep, technical support in the field as well as in the “factory”, marketing, general management, technical writing and other areas. I will share with attendees the types of opportunities that one can enjoy working in an instrument company environment. I found it very enjoyable to work alongside of other R/D and applications chemists, mechanical, electrical and software engineers, technical writers, quality engineers, finance and marketing staff where a diverse “team” approach can accomplish more than individuals working alone. Tackling problems from different angles can enrich one’s appreciation for other fields of endeavor. If one likes the opportunity to travel, scientists are encouraged to participate in local, national and international symposia and joint projects with academia and customer “real life” projects. Publications and technical presentations are also encouraged, just as in academia. At the end of this presentation, attendees should have a better understanding of opportunities available in the instrumentation industry.

Keywords: Chromatography, Education, Separation Sciences
Application Code: Other
Methodology Code: Education/Teaching
In a way, a career is similar to telling a story. A story - your story - is likely to be personal and unique. This presentation will highlight my experiences - spanning 35 years - in academia, industry, and business. My story. But a story that can also be told by colleagues, friends, and family. Unique perspectives gained in the sciences as well as the ability to lead - whether oneself or others - with the use of time, a value for relationships, and a preference for responsibilities that evoke passion. My hope is that participants will be inspired to find their own story and have the courage to share it with others - everyday.
The landscape of bioanalysis has been changed significantly in the past decade. Bioanalysis continues playing important roles in supporting the entire drug discovery/development by providing pivotal PK/PD data for go/no-go decision. Additionally, bioanalysis currently also provides inputs to project team on optimization compound properties. Bioanalytical scientists continue applying new technology for better, quicker and more sensitive analysis. Beyond bench-work, bioanalytical scientists act as good program managers and study monitors for work done externally at Contract Research Organizations (CROs) and other collaborators. Due to the time and budget constraints, bioanalytical scientists should exercise the right vigor for balancing compliance and cost-effectiveness. Finally, bioanalytical scientists also need to demonstrate Credo behavior and be a good ambassador to our communities. This presentation will highlight the current work landscape and behavior requirement of bioanalytical scientists working at bioanalytical and pharmacokinetic department at Janssen Pharmaceutical R&D, Pharmaceutical Companies of Johnson & Johnson.

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

Application Code: Pharmaceutical

Methodology Code: Liquid Chromatography/Mass Spectrometry
In this presentation, scanning electrochemical microscopy will be used to investigate ion permeability of the nuclear envelope, which separates the nucleus of a biological cell from the cytoplasm. Ion transport through the nuclear envelope is mediated by the nuclear pore complex. We will apply a small electrode probe with a diameter of 1 um to investigate ~25 pores. We will reveal how the permeability of the pores depend on the charge and hydrophobicity of transported redox molecules. This information is crucial to assess the electrostatic and hydrophobic characters of transport barriers of the nuclear pore complex.
Hyphenated techniques in analysis offer the potential to provide far greater information than a single detection technique. Here we combine electrochemical sensors with fluorescence microscopy, via the use of transparent electrodes, for understanding the response of mammalian cells to stimuli. Initially, electrode surfaces are modified with self-assembled monolayers to control the adhesion of cells to the surfaces. The position of the cell-adhesive ligands, and how the focal adhesions form as a result of the ligand distribution is first characterized by single molecule localisation microscopy. Thereafter, fluorescent microscopy combined with electrochemical impedance is employed to not only determine how the cells respond to therapeutics that target the G-Protein Receptors, but also the timescales of downstream signalling events as a result of these stimuli. The application of this technology for understanding anti-fibrotic drugs action is then demonstrated before finally showing how the impedance changes of single cells can be measured.

**Keywords:** Bioanalytical, Biosensors, Drug Discovery

**Application Code:** Bioanalytical

**Methodology Code:** Electrochemistry
Simultaneous and Selective Detection of Two Molecules with a Single Solid-Contact Potentiometric Ion-Selective Electrode

A polymeric membrane ion-selective electrode (ISE) is typically designed for determination of one specific ion using conventional method. In this presentation, we demonstrate a simple, versatile and sensitive platform for simultaneous detection of two molecules with a single ISE. Under a series of periodic galvanostatic polarization, a solid-contact ISE without ion exchanger properties under zero-current conditions has been successfully used for simultaneous detection of two opposite charged ions with high sensitivity, good selectivity and fast reversibility. By integration of biorecognition elements with the potentiometric measurement, highly sensitive and selective detection of a broad range of different molecular targets can be predicted. As a proof of concept, a potentiometric genosensor based on magnetic beads-enzyme sandwich assay has been designed for sensitive and selective detection of pathogenic bacteria Escherichia coli 0157:H7 and Staphylococcus aureus. Under optimal conditions, two bacteria nucleic acid sequence can be detected simultaneously with high sensitivity and good selectivity by using a single solid-contact potentiometric ISE. The detection limits of Escherichia coli 0157:H7 DNA and Staphylococcus aureus DNA are 120 fM and 54 fM (3), respectively. In spite of its simplicity, this potentiometric technique based on ISE can be an attractive tool to perform two or more analytes measurements.

Keywords: Biosensors, Contamination, Detection, Electrodes
Application Code: Bioanalytical
Methodology Code: Sensors
Strategies to prepare synthetic receptors based on molecularly imprinted polymers (MIPs) and aptamers for selective recognition of proteins will be presented. The practical applicability of both kind of receptors depends largely not only on the proper design of the synthetic route but also on the physical chemical properties of the targeted species and their practically relevant concentrations. We will show that while MIP-based sensors have a clear niche in quantifying high abundance proteins in a clinical setting, aptamers can be used for single species detection as exemplified through the quantitative assessment of albumin in urine [sup]1[/sup] and respiratory syncytial virus counting in human sputum samples, [sup]2[/sup] respectively.

Acknowledgment. This work was supported by the Lendület program of the Hungarian Academy of Sciences (LP2013-63) and ERA-Chemistry (2014, 61133; OTKA NN117637)

References:

Keywords: Electrochemistry, Electrodes, Imaging, Sensors
Application Code: Bioanalytical
Methodology Code: Sensors
Solid-contact ion-selective electrodes (SC-ISEs) are miniaturized ion sensors by omitting inner filling solutions. By using the technology of SC-ISEs, electrolyte cartridges for measuring electrolytes in blood such as Na, K and Cl can be miniaturized. To measure these electrolytes, a cartridge requires not only ISEs for target ions but also a reference electrode that generates constant electric potential. A silver/silver chloride electrode is usually used as a reference electrode together with potassium chloride (KCl) solution as reference solution. A junction potential between sample solution and reference solution becomes small due to similar mobility of K and Cl ions. Lithium acetate (LiAcO) solution is also known as a solution with small junction potential due to similar mobility of Li and AcO ions. Combining a lithium-selective ISE and LiAcO solution as a reference solution, it should work as a reference electrode. Since either ions (Li or AcO) are not usually target ions, contamination of reference solution into sample solution ideally does not affect measurement values. To demonstrate this new concept, we made prototype of electrolyte cartridges. The cartridge consists of Na, K and Cl selective electrodes for target ions and Li-selective electrodes as a reference electrode. Na, K and Li electrodes are solid-contact ISEs using intercalation compounds (sodium nickel hexacyanoferrate, potassium nickel hexacyanoferrate and lithium iron phosphate, respectively) as solid contact. Use of 1M LiAcO as reference solution, the cartridge showed good slopes (>58mV/decade).
Wearable sensor technologies play a significant role in realizing personalized medicine through continuously monitoring an individual’s health state. To this end, human sweat is an excellent candidate for non-invasive monitoring as it contains physiologically rich information. In this talk, I will present our recent advancements on fully-integrated perspiration analysis system that can simultaneously measure sweat metabolites, electrolytes and heavy metals, as well as the skin temperature to calibrate the sensors' response. Our work bridges the technological gap in wearable biosensors by merging plastic-based sensors that interface with the skin, and silicon integrated circuits consolidated on a flexible circuit board for complex signal processing. This wearable system is used to measure the detailed sweat profile of human subjects engaged in prolonged physical activities, and infer real-time assessment of physiological state of the subjects.
A calibration-free ion-selective electrode measurement requires that only the phase boundary potential at the sample/sensing membrane interface is variable (depending on the activity of the target ion in the sample) while all the other phase boundary potentials in the electrochemical cell consisting of the indicator electrode, the reference electrode, and the sample are constant. Historically, the biggest difficulties lie in establishing a reproducible phase boundary potential at the interface of the sensing membrane and the underlying electron conductor. This talk will address our newest finding using ion-selective membranes doped with redox buffers. It will also address the question on how water affects this phase boundary potential and how the time necessary for conditioning in aqueous sample solutions can be minimized.

Keywords: Bioanalytical, Ion Selective Electrodes, Potentiometry
Application Code: Biomedical
Methodology Code: Electrochemistry
Solid state potentiometric sensors have an inherent advantage of miniaturization. We developed a carbon based solid state -Ion Selective Electrode (ISE) sensors to detect a wide array of analytes such as Ca²⁺, H⁺, and NH₄⁺. These unique low PVC content sensors are capable of performing amperometric measurements and then could be switched to potentiometric measurements. The 35-40 [μm] diameter micro-ISE showed fast response time (~1 sec or less), low limit of detection (~1 [μM]) and broad linear range (low [μM] to high mM) and thus ideal to be used as a chemical probe in scanning electrochemical microscopy (SECM) for high-resolution chemical imaging. In addition, we also developed 100 [μm] diameter solid-state flexible wire sensors to detect pH and Ca²⁺ at the interface of biomaterials and biofilms. Recent applications of these unique microsensors to study microbial metabolism will be presented in the conference.

This work was supported by National Institute of Dental and Craniofacial Research (NIDCR), NIH (Grant # R21DE025370).
A current trend of bioanalysis is for deeper understanding of life phenomena and practical application to medical and pharmaceutical fields. To analyze the biological events in living cells, technologies related to fluorescence and bioluminescence imaging have been advancing rapidly in the past decade. Remarkable advancement has been achieved in the monitoring of bioactive small molecules, protein conformational changes, protein localization and dynamics, and protein-protein interactions in real-time at the level of single living cells and organisms. I herein focus on new bioluminescent sensors using luciferases for the analysis of intracellular signaling and cellular communications; the principle is based on complementation or reconstitution of split-luciferase fragments when they are brought sufficiently close together. To show the applicability, I first demonstrate direct monitoring of GPCR-arrestin interactions in living cells. The split luciferase fragments enabled to temporally sense different kinds of GPCR activities and to compare differences of arrestin subtypes. The practical applicability was demonstrated for large chemical library screening to identify specific or biased inhibitors. Another application of split-luciferase technologies is to monitor cell-cell fusions. We designed the luciferase fragments reconstitution by protein splicing and used it for detecting myogenesis promoting or inhibiting effects by chemical compounds in C2C12 cells. The luminescence intensities increased by the treatment of myogenesis-promoting reagents and decreased by a myogenesis inhibitor, TNF-alpha. Using the luciferase fragments, we identified some myogenesis-promoting compounds by chemical library screening. These techniques will engender deeper understanding of biological systems and will break new ground in pharmacological analysis.


Keywords: Bioanalytical, Biopharmaceutical, Imaging, Luminescence
Application Code: Bioanalytical
Methodology Code: Fluorescence/Luminescence
Selective imaging of targeted cells in living samples with chemical probes remains highly challenging. LacZ gene, which encodes Escherichia coli [beta]-galactosidase, is widely used as a marker for cells with targeted gene expression or disruption. However, it has been difficult to detect lacZ-positive cells in living organisms or tissues at single-cell resolution, limiting the utility of existing lacZ reporters. Here we present a newly developed fluorogenic [beta]-galactosidase substrate, SPiDER-[beta]Gal-1, which is suitable for labeling live cells in culture, as well as in living tissues and organisms. This precisely functionalized fluorescent probe based on intramolecular spirocyclization and quinine methide chemistry exhibited dramatic activation of fluorescence upon reaction with the enzyme, remains inside cells by anchoring itself to intracellular proteins, and provides single-cell resolution. Neurons labeled with this probe preserved spontaneous firing, which was enhanced by application of ligands of receptors expressed in the cells, suggesting that this probe would be applicable for investigating functions of targeted cells in living tissues and organisms.

Keywords: Bioanalytical, Biosensors, Fluorescence, Imaging
Application Code: Bioanalytical
Methodology Code: Fluorescence/Luminescence
A microfluidic device based on ionic current sensing system for high-throughput and practical single bacteria and mammalian cell sizing was developed, and furthermore, discrimination of bacterial species and mammalian cell deformability was achieved. The highly precise sizing system based on blocking ionic current at narrow microchannel provided the information on antibiotic resistant strains of bacteria. Deformability changes associated with passage of adipose tissue-derived stem cells (ASCs) were also successfully detected by the device without any chemical or biological modification. The mechanical properties of a cell are extremely important because changes in the mechanical properties are indicative of diseases ranging from diabetes to malignant transformation. Considering the heterogeneity within a population of cancer cells and stem cells, a robust measurement system at the single cell level is required in both research and clinical situations. Recent developments in microfluidic devices have advanced the throughput of mechanophenotyping measurements. However, since most of these assay techniques essentially rely on optical detection systems, the spatial resolution was limited to a few μm in the xy plane and less in the z direction. We have proposed the microfluidic device with two consecutive constrictions for a single cell sizing and deformability measurements based on blocking ion current. In this work, we validated the methodology and expanded the application field to stem cell research.
Fluorescence polarization (FP) is a versatile solution-based method that enables the study of molecular interactions such as protein-protein, protein-DNA, and protein-ligand binding interactions. Since this method is rapid and easy to implement, it is used in clinical and biomedical applications. In particular, fluorescence polarization immunoassay (FPIA) that combines FP and competitive immunoassay is a well-established technique for monitoring of therapeutic drugs in the blood and quantitative analysis of drug residues in foods.

Recently, we developed a unique FP measurement system based on the synchronization between the orientation of the liquid-crystal molecules of a LC display and the sampling frequency of an image sensor to realize simultaneous FP analysis of multi-samples.

The feature of our system developed here is to be able to acquire a 2D image of FP. By this feature, FP of multiple samples on the image can be acquired simultaneously. Using the system, we demonstrated the multisample FPIA of prostaglandin E2.

**Keywords:** Fluorescence, Immunoassay, Lab-on-a-Chip/Microfluidics, Liquid Crystal

**Application Code:** Food Safety

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

Many applications in chemistry, materials research, and biomedicine simultaneously require the chemical information and thermophysical parameters (thermal conductivity/diffusivity, heat capacity, etc.). Photothermal spectroscopy (PTS) is capable for the direct assessment of low light absorptions of various materials. However, PTS also develops as thermal spectroscopy as the signal is governed by the heat redistribution in the entire system. Coupling thermal and optical features of PTS is a base for spectroscopy/sensing/imaging for materially or optically heterogenous systems.

PTS techniques are classified relative to the timescale of underlying processes. Thermal-lens spectrometry (TLS) corresponds to thermal stationary states of milliseconds to seconds scale, which coincides with many chemical and photochemical processes. Thus, TLS is advantageous for chemical analysis and estimating heat-transfer parameters in solutions including disperse systems.

The estimation of subnanogram amounts of light-absorbing dispersed particles (carbon nanomaterials and proteins) by TLS will be shown and discussed. The detection limits are 20-fold lower than for conventional absorption measurements.

An approach is proposed for TLS of nanoparticle dispersions based on due account of heat transfer inside a particle followed by heat redistribution to the bulk (Fig. 1). The deconvolution of the transient curve makes it possible to describe the thermal equilibrium and to assess the parameters of the disperse phase. The data corresponding to thermal equilibria show good precision. Two TLS techniques for nanodiamonds—transient and imaging—results in concordant values. Along with techniques for size and heat capacity of disperse systems (DSC, DLS, XRD, etc.), this approach was applied for thermal diffusivity and thermal conductivity of aqueous dispersions of carbon nanomaterials and protein complexes.

Figure 1. heat transfer in a disperse system of light absorbing nanometer-size objects.

Keywords: Bioanalytical, Instrumentation, Material Science, Molecular Spectroscopy
Application Code: Material Science
Methodology Code: Molecular Spectroscopy
When an aqueous solution is frozen, pure ice crystals and freeze-concentrated solution (FCS) are separated. The FCS exists in the grain boundaries (GBs) between ice crystals. The GBs formed on the surface of sucrose-doped ice have channel-like structures. The channel width is in the micro/submicrometer range when the initial sucrose concentration is as low as 10-100 mM. Because the volume of FCS is a function of the sucrose concentration and temperature, we can control the size of the GB channel by changing these parameters. This size-tunability of the GB channel enables us to achieve size-selective separation of nano- and micromaterials. For example, substances travelling along the GB channel undergo larger physical interference from the ice wall as temperature decreases. We utilize this concept to achieve size-selective separation by managing the physical interaction with the ice wall.

An ice-platform was fabricated by freezing aqueous sucrose containing samples (microparticles, DNA etc.) in a copper cell equipped with two Ag-AgCl electrodes. Samples were spontaneously introduced in the GBs since they are excluded from pure ice crystals. Voltage was applied to the electrodes, and the electrophoretic migration of samples in the GB was measured under a fluorescence microscope.

When 75 mM aqueous sucrose is frozen at -9.0°C, the GB channel width was ca. 1 [micro]m. The channel width on this platform can be varied in a range 0.2-3 [micro]m by changing temperature. The migration of polystyrene particles with various sizes were measured in the GB at various temperatures. Smaller particles start to migrate at a lower temperature when the temperature increases. The size-tunability of the GB channels has thus been confirmed. We have also determined ice zeta potential, which plays an important role in the determination of migration in the GB channels. Zeta potentials under various conditions will also be discussed to reveal the nature of the ice/FCS interface.

Keywords: Electrophoresis, Nanotechnology, Separation Sciences
Application Code: Nanotechnology
Methodology Code: Separation Sciences
Remote Measurement of Aerosol Surface Tension

Thermal fluctuation induces capillary waves at a liquid interface. Capillary waves have tiny amplitude (typically a few Ångstroms) and random wavenumbers. A quasi-elastic light scattering (QELS) method measures Doppler shift during laser light scattering by the capillary wave. In our previous reports, spontaneous resonance of thermally-induced capillary wave has been demonstrated on liquid surfaces with spatially confinement by a microchannel [1] and by a circular aperture [2]. In these experiments, capillary waves satisfying the resonant condition keep oscillating, and those in non-resonant condition disappear soon. Then, characteristic peaks corresponding to the resonance appear in QELS power spectrum. From the peak frequencies, surface tension has been available.

In this study, we extend our QELS method for spatially-restricted interfaces to aerosols. When optically trapped aerosols were measured with QELS method, we observed resonant peaks in the spectra, which can be assigned to spontaneous spherical resonance of thermally induced aerosol surface deformation. Then, we tried to measure surface tension of aerosol containing organic surface-active compounds.


Keywords: Aerosols/Particulates, Lab-on-a-Chip/Microfluidics, Light Scattering, Spectroscopy
Application Code: Environmental
Methodology Code: Microfluidics/Lab-on-a-Chip
# Organized Contributed Sessions

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**Primary Author**
Shinichi Enami  
NIES, Japan

**Abstract Text**

Products and intermediates of the prompt reactions of a variety of aqueous species initiated by gas-phase oxidants such as hydroxy radicals (OH radicals) at the gas-liquid interfaces are detected by a newly-developed mass spectrometry under ambient conditions. Exposure of submillimolar reactant aqueous microjets to gas-phase reactants yielded interfacial species, including peroxyl radicals (RO2 radicals), that are simultaneously identified in situ by mass spectrometry. Atmospheric and environmental implications are discussed.

**Keywords:** Aerosols/Particulates, Environmental Analysis, Environmental/Air, Environmental/Water  
**Application Code:** Environmental  
**Methodology Code:** Mass Spectrometry
According to the World Health Organization, antimicrobial resistant (AMR) bacterial infections cause approximately 700,000 deaths per year and are predicted to be the number one cause of death worldwide by 2050 unless significant steps are taken (1). It is not only imperative to develop new antibiotics, but to develop faster and better diagnostic methods allowing the patient to receive the correct treatment as soon as possible and minimize indiscriminate use of antibiotics that ultimately lead to further resistance. Our group recently reported a paper-based analytical device (PAD) that detects bacteria resistant to penicillins and cephalosporins, the most commonly prescribed antibiotics, using a simple colorimetric indicator that turns from yellow to red in the presence of these bacteria based on enzyme expression (Figure 1A). This test was demonstrated with sewage water and various bacterial species that do and do not express AMR, showing 98% accuracy (2). Here, we report expansion of this test to include detection of bacteria resistant to carbapenems, a commonly used class of last-resort antibiotics. If bacteria in the sample are resistant to carbapenem antibiotics, they will hydrolyze imipenem, a carbapenem antibiotic, resulting in a corresponding decrease in pH from the hydrolysis (Figure 1B). This reaction takes place in the presence of multiple pH indicators on the device to indicate hydrolysis to the end-user. The PAD has been verified using a model enzyme system, and will soon be tested with bacterial isolates. This new PAD has so far demonstrated a successful expansion of our previous device, exhibiting a more comprehensive AMR detection method.

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References

Flow Cytometric Analysis of a Paper-based Tumor Model to Investigate Chemotherapeutic Effectiveness

In vitro assays designed to predict the efficacy of new cancer chemotherapeutics rely on two-dimensional (2D) cultures. While these assays provide simplified readouts, they are reductionistic and fail to recapitulate the diffusion-limited conditions in tumors. These conditions often lead to regions of nutrient and oxygen deficiency, which promote the development of a chemoresistant phenotype. To better identify chemotherapeutics that target these chemoresistant populations, we engineered a three-dimensional paper-based culture (PBC) consisting of cell-laden paper scaffolds stacked together to form a tissue-like construct. By controlling nutrient flux into the PBCs, diffusion-limited gradients yield conditions similar to those within poorly vascularized tumors. Modulating the cell density allows us to experimentally control the formation of these gradients, providing distinct chemical environments within each scaffold. After prolonged incubation in the presence of a chemotherapeutic, the cultures can be easily disassembled into individual scaffolds and analyzed in a spatially resolved manner. In this work, cells will be exposed to SN-38—the cytotoxic metabolite of the chemotherapeutic prodrug irinotecan—for 48 hours then extracted from individual scaffolds. Flow cytometry will be used to identify the percent of viable, senescent, and dead sub-population of cells per scaffold, mapping localized responses to SN-38. The extracted populations will be compared across different scaffolds, allowing us to assess the effectiveness of SN-38 under a range of environmental conditions. By integrating oxygen sensors into these cultures, we can quantify the oxygen gradients across the PBC in real-time and make direct comparisons between local oxygen tensions and cellular chemoresistance. This work will highlight PBCs as an enabling technology that can predict effective chemotherapeutics while also identifying extracellular mechanisms which affect therapeutics.

Keywords: Bioanalytical, Clinical/Toxicology, Drug Discovery, Lab-on-a-Chip/Microfluidics
Application Code: Bioanalytical
Methodology Code: Microfluidics/Lab-on-a-Chip
Bioanalytical - Microfluidics/Lab-on-a-Chip

3D-Printed Rotational Manifold Designed for the Rapid Colorimetric Detection of Salmonella

In the United States, Salmonella is responsible for more hospitalizations and deaths per year than any other pathogen.[sup]1[/sup] Salmonella outbreaks are typically linked to agricultural sources such as poultry or raw vegetables. Current detection methods are laborious and require a central laboratory, making in-field detection expensive and time-consuming. Therefore, a rapid, sensitive, and field-deployable Salmonella assay is critical to prevent deadly outbreaks. We have developed a sandwich immunoassay utilizing immuno-magnetic separation (IMS) and chlorophenol red-D-galactopyranoside for Salmonella detection on a paper-based analytical device (PAD); however, the assay required multiple steps and laboratory equipment, making field measurements difficult.[sup]2[/sup] To overcome these limitations in agricultural or resource-limited settings, we designed a reusable 3D-Printed manifold that couples with disposable PAD layers for reagent delivery and detection (Figure 1). The reagents are delivered to the sample by rotating the reagent layer so buffer is dispensed through the channel and sample layers. IMS is accomplished with a magnet fitted directly underneath the sample layer and a colorimetric response indicates the presence of Salmonella, which is quantified visually or with imaging software. Each test uses one reagent layer and one sample layer, while the manifold is re-useable. The reagent and sample layers are portable, disposable, and inexpensive, making them ideal for field work, and the manifold is designed to streamline the assay for minimized training. In addition to our Salmonella assay, this device could be used to simplify other complicated immunoassays and bring them from the laboratory to the field.

All funding was provided by the USDA through the National Wildlife Research Center

2. Srisa-Art M. et al., [i]In Prep. Analytical Chemistry[/i] [b]2017[/b]

Abstract Text

In the United States, Salmonella is responsible for more hospitalizations and deaths per year than any other pathogen.[sup]1[/sup] Salmonella outbreaks are typically linked to agricultural sources such as poultry or raw vegetables. Current detection methods are laborious and require a central laboratory, making in-field detection expensive and time-consuming. Therefore, a rapid, sensitive, and field-deployable Salmonella assay is critical to prevent deadly outbreaks. We have developed a sandwich immunoassay utilizing immuno-magnetic separation (IMS) and chlorophenol red-D-galactopyranoside for Salmonella detection on a paper-based analytical device (PAD); however, the assay required multiple steps and laboratory equipment, making field measurements difficult.[sup]2[/sup] To overcome these limitations in agricultural or resource-limited settings, we designed a reusable 3D-Printed manifold that couples with disposable PAD layers for reagent delivery and detection (Figure 1). The reagents are delivered to the sample by rotating the reagent layer so buffer is dispensed through the channel and sample layers. IMS is accomplished with a magnet fitted directly underneath the sample layer and a colorimetric response indicates the presence of Salmonella, which is quantified visually or with imaging software. Each test uses one reagent layer and one sample layer, while the manifold is re-useable. The reagent and sample layers are portable, disposable, and inexpensive, making them ideal for field work, and the manifold is designed to streamline the assay for minimized training. In addition to our Salmonella assay, this device could be used to simplify other complicated immunoassays and bring them from the laboratory to the field.

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2. Srisa-Art M. et al., [i]In Prep. Analytical Chemistry[/i] [b]2017[/b]

Keywords: Bioanalytical, Food Safety, Immunoassay, Lab-on-a-Chip/Microfluidics

Application Code: Bioanalytical

Methodology Code: Microfluidics/Lab-on-a-Chip
Integration of Dielectrophoretic Selective Single-Cell Capture at a Wireless Electrode Array with On-Chip Analysis of Single Circulating Tumor Cells

Due to the extreme rarity and heterogeneity of circulating tumor cells, the development of versatile selective isolation techniques and subsequent single-cell analysis continues to be an important challenge. Separation based on dielectrophoresis features antibody-independent separation and also avoids leukocyte contamination. However, many of the current approaches suffer from low throughput and are not amenable to on-chip single-cell analysis. These limitations stem from design constraints such as the requirement that all electrodes must be connected via wire leads to the power source. Further, in DEP devices that employ insulating posts to shape the electric field, integration of these structures intended for cell capture with other features, such as chambers for on-chip analysis, is non-trivial. Here we report a DEP strategy that addresses these concerns. First, the use of arrays of bipolar electrodes removes the requirement of ohmic contact to individual array elements, thus enabling otherwise unattainable device formats. Second, pockets extruding from either side of the microchannels render the design applicable for high-fidelity single-cell capture. Third, extension of each pocket into reaction chambers allows the retention and interaction of single cells and PCR reagents. We anticipate that this new DEP technique will enable on-chip genetic analysis of individual circulating tumor cells.

Keywords: Bioanalytical, Detection, Lab-on-a-Chip/Microfluidics, Separation Sciences
In tumors, rapid proliferation and aberrant vasculature leads to a diffusion dominated environment containing gradients of nutrients, oxygen, pH, and cellular waste products. Cancerous cells adapt to these stressed environments, resulting in cell populations that are drug resistant and invasive. Gradients of abiotic factors and signaling molecules found in tumors promote and direct the movement of these invading cells. Paper-based assays are an emerging platform to study cellular invasion in gradients found in the tumor microenvironment. Our paper-based invasion assay utilizes a single sheet of paper, wax-patterned to contain channels of cellulose fibers surrounded by hydrophobic borders, in which cell-laden hydrogels are seeded. By sandwiching the paper scaffolds between materials that limit exchange with fresh culture medium, we are able to generate defined gradients of oxygen and pH across the length of the channel. To map evolving oxygen and pH gradients in real-time, we developed a dual oxygen/pH sensor. This two-layer sensor is fabricated by coating a ratiometric pH sensing film on top of a luminescent oxygen sensor previously developed in our lab. The resulting film is sensitive in regions with low oxygen tension and near-neutral pH. Using fluorescent micrographs we are able to simultaneously image small molecule gradients and cellular invasion. Given that hypoxia and acidosis cooperatively direct invading cells, we studied the invasion of MDA-MB-231 cells along these gradients. With this sensor, we are able to tease out the drivers of invasion by modulating the steepness of abiotic factor gradients.

Keywords: Bioanalytical, Biosensors, Imaging, Lab-on-a-Chip/Microfluidics
Application Code: Bioanalytical
Methodology Code: Microfluidics/Lab-on-a-Chip
The Development of Automated Microfluidic Droplet System for Dynamic Cellular Signaling Research with High Temporal Resolution

Microfluidics is becoming a powerful tool in biological research, enabling low-abundance protein detection and dynamic study of cells with high temporal resolution. Droplet-based microfluidics can encapsulate samples in water-in-oil emulsions to reduce analytical volumes to the picoliter to nanoliter levels, making it extremely useful in single-cell analysis and high-throughput analysis. However, most droplet fluidics assays have been limited to sample preparation digital readout. Heterogeneous immunoassays, such as ELISA, still require complicated workflows to accomplish on-chip. Here we report the development of a device for fully automated droplet generation and analysis based on pressure driven microvalves for high-resolution, quantitative cell secretion sampling. The first-generation device (Figure) is composed of a 3D-templated reservoir for tissue culture, a “Y-shape” channel for reagents and sample mixing, a “T-junction” channel for droplet formation by aqueous and oil pumps, a reference channel to overcome long-term fluorescence fluctuations, and a long droplet storage channel allowing assay incubation and fluorescence measurement. The in-house written LabVIEW application allows automation of the device and precise control over several droplet parameters such as size, spacing, and ratio of sample and reference droplets. By integrating a homogeneous immunoassay on-chip, insulin secretion quantification from single pancreatic islets was achieved with 15-second temporal resolution (Figure). In the second-generation device, one more inlet was included for generic enzyme assay reactions (glucose and glycerol assays validated), and an S-shape oil micropump was added to improve droplet control and sampling rate. Since enzyme assays are broadly applicable to detect cellular molecules, this device should be readily transferable to a variety of proteins and small molecule secretion dynamic study from many cell types.

Keywords: Bioanalytical, Fluorescence, Immunoassay, Lab-on-a-Chip/Microfluidics
Application Code: Bioanalytical
Methodology Code: Microfluidics/Lab-on-a-Chip
Entrainment of Pancreatic Islets of Langerhans with Cholinergic Waveforms

Pulsatile insulin secretion into the portal vein is crucial for efficient glucose homeostasis and is achieved through entrainment of millions of pancreatic islets of Langerhans into a common oscillatory phase. It has been established that the stimulation of ß-cells with acetyl choline or the cholinergic agonist, carbachol, promotes transient synchronization of glucose-induced intracellular Ca2+ ([Ca2+]i) oscillations in islets. However, all reports to date only show the short term synchronizing effects that single pulses of cholinergic agonists have on islets. Our goal is to explore the possibility of entraining glucose-induced [Ca2+]i oscillations in islets to the frequency of periodic pulses of carbachol.

Using a piezoelectric pressure-driven perfusion system, a microfluidic device was developed to stimulate murine islets with various carbachol pulse patterns while minimizing band broadening of the pulses. The pulse duration, magnitude and the inter-pulse rest periods were varied while islet activity was measured by fluorescence imaging of [Ca2+]i using Fura-2. It was found that a variety of carbachol patterns entrained and synchronized islets. A stimulation profile with 5 minute periods of 10-second-long pulses of 10 uM carbachol entrained islets to the same frequency with a phase offset of 146.7 ± 2.2%. Other carbachol pulse profiles also entrained islets and promoted islet synchronization all within 20% of mean phase offset values.

These results provide experimental evidence of the importance of periodic parasympathetic signals and their ability to mediate pulsatile insulin secretion from islets. Such findings may contribute new insights into combating glucose intolerance that preceded type 2 diabetes.

Keywords: Bioanalytical, Lab-on-a-Chip/Microfluidics, Medical
Application Code: Bioanalytical
Methodology Code: Microfluidics/Lab-on-a-Chip
Bioanalytical - Microfluidics/Lab-on-a-Chip

Integrated Bioreactor and Glucose Measurement to Determine Effect of Pulsatile Hormone Perfusion on Hepatocytes

In type II diabetes, defective hormone secretion from the pancreas causes abnormal production and storage of glucose by the liver. To maintain glucose homeostasis, insulin is released in ~6 min pulses in vivo, but how these pulses affect glucose management by the liver is still unclear. In this study, a microfluidic system was developed to determine the effect of dynamic insulin levels on glucose regulation by a human hepatocyte cell line, HepG2.

Pulses of insulin were delivered to HepG2 cultured in a 60-uL microfluidic bioreactor made of polydimethylsiloxane (PDMS), that contained a microchamber for 3D cell growth. To ensure insulin pulses were consistent throughout the device, the fluid flow and pulses were modeled with finite element analysis. The simulation results indicated that there was minimal broadening of the pulses throughout the device. Insulin was then delivered in either 1-minute pulses, every six minutes, or was delivered in a constant manner at the same dose.

Resulting glucose output was measured with an enzymatic glucose assay that produced a fluorescent readout. The insulin pulses stimulated decreases in measured glucose concentration with a consistent temporal correlation to the insulin pulses, indicating production of glycogen. In the future, to minimize longitudinal broadening of samples upon mixing with assay reagents, a droplet-based system will be incorporated to fractionate the samples and to decrease reaction times as a result of better mixing.

Keywords: Analysis, Bioanalytical, Enzyme Assays, Lab-on-a-Chip/Microfluidics

Primary Author: Anna G. Adams
Florida State University

Co-Author(s): Jose L. Mendoza-Cortes, Michael G. Roper, Radha Krishna B. Raja
Capillary electrophoresis (CE) is an important analytical separation method that has found many applications such as clinic diagnosis, food analysis, and environmental monitoring. Application of CE for trace analysis is often limited by the concentration detection limit. We have developed an in-line electrophoretic preconcentration method that, prior to CE, continuously stacks analytes using isotachophoresis (ITP) without the volume limitation of the separation column. The separation column of a 50 cm-long capillary is composed of two sections: 4 cm-long polyacrylamide monolith column for ITP preconcentration and the remaining 46 cm-long open column for CE separation. The monolith is selectively formed at one end of the capillary using UV-initialized polymerization. ITP within the monolith presents a number of advantages, including lower dispersion of stacked samples, no dead volume from the connectors and better operational control. Continuous stacking in ITP is implemented by applying a counter flow in the opposite direction to analyte electrophoretic migration so that analytes are stacked at a stationary position in the monolith section. The counter flow is generated by applying pressurized nitrogen in a well-sealed anode electrolyte cell prepared via 3D printing. The performance of the comprehensive ITP-CE system is demonstrated by separating oligosaccharides.

Keywords: Bioanalytical, Capillary Electrophoresis, Instrumentation, Separation Sciences
Application Code: Bioanalytical
Methodology Code: Capillary Electrophoresis
The control of the electroosmotic flow (EOF) in capillary electrophoresis is paramount to a good separation. A broad range of techniques are available to alter both the magnitude, and direction of the EOF, typically through surface modifications of the capillary, and additives to the separation buffer.

One class of modifiers, phospholipids, have been shown to be effective as coatings for biological separations, and able to be tuned for normal or reverse polarity EOF through the addition of calcium to the separation buffer. We have recently shown that other divalent and trivalent cations can be included in the separation buffer, rapidly displacing the calcium from the bilayer, and altering the magnitude of the EOF.

In this work we go a step further, employing the facile displacement of the divalent cations to switch the direction of the EOF mid-separation. By using a discontinuous buffer system, where one buffer contains calcium, and the other does not, we can control the initial direction of the separation, and cause EOF reversal partway through the separation. We will present the conditions for such manipulations, our modeling of the effect, and demonstrate how this may be applied for large volume injections and sample stacking.

Keywords: Bioanalytical, Capillary Electrophoresis, Electrophoresis, Separation Sciences

Application Code: Bioanalytical
Methodology Code: Capillary Electrophoresis
Sampling is a predominant method used to study interstitial fluids in living tissues. Low-flow push-pull perfusion is a technique which provides the ability to collect a sample at the tip of a probe in a specific tissue volume precisely. Recently, reductions in tip diameter to an average of 30 micron has been reported, but further reductions to less than 10 micron provides still more spatial precision. The focus of this talk is to describe the construction and testing of these pulled LFPP probes, as well as in vivo amino acid concentration data collected by LFPP probes. The probes were constructed of 75/150 micron ID/OD micrometer capillary tubing threaded through 180/360 micron capillary tubing. These concentric capillaries are pulled to a desired diameter in a butane flame. Probe tips were then measured through optical and electron microscopy. Probes were calibrated to desired withdrawal flow rates using a vacuum pump and syringe pump settings for infusion flow rates, followed by sample recovery experiments of test molecules. Probes were placed into retinal tissues for collection of extracellular fluid. Amino acids in the sample were separated using CE, then analyzed through LIF and MALDI. Using scanning electron microscopy, probe tip diameter was found to be an average of ~6 micron, and possess more uniform tip shapes compared to previous findings. Vacuum pressures needed for 10 nL/min flow were similar to previously published LFPS data. Sample recovery exceeded 95% for all test molecules. With appropriate infusion protocols during placement, probes insertion into retinal tissue allowed for sample collection. Amino acid levels were qualitatively and quantitatively similar to previously published work. The method of producing pulled LFPP probes was improved upon by eliminating a step to cut back the probe tip. The consistency of the miniaturized LFPP probes, paired with the techniques for the prevention of clogging, allowed for reliable sampling of in vivo tissue.
Capillary Electrophoresis

Micellar Electrokinetic Chromatography for Measurement of Dopamine and D-serine Secretion from Human Islets of Langerhans

N-methyl-D-aspartate (NMDA) receptors, which use D-serine (D-Ser) as a co-agonist, and Ser racemase, the enzyme responsible for the enantiomeric conversion of L-Ser to D-Ser, have been observed in human pancreatic islets. The neurotransmitter dopamine (DA), and its related enzymes, are known to be present in human islets as well. However, neither the secretion profile of DA from glucose stimulation is known, nor whether D-Ser is secreted from islets.

In this study, a two-surfactant micellar electrokinetic chromatographic (MEKC) method capable of resolving DA and D-Ser from other small molecules was used to determine if they are released upon glucose stimulation. The chiral surfactant, sodium deoxycholate (SDC), was added to a conventional sodium dodecyl sulfate (SDS) MEKC buffer to resolve D- and L-Ser with minimal loss of resolution of other analytes. Through the use of both surfactants, Ser enantiomers were baseline resolved with DA resolved from other amino acids when derivatized with 2,3-napthalenedicarboxaldehyde. Optimized buffer conditions were 150 mM boric acid (pH 9.2), 45 mM SDS, and 35 mM SDC on a 60 cm capillary with 25 [micro]m ID.

Calibration curves showed LODs of 300 nM for DA and 2 nM for D-Ser. The method was applied to examine release from human islets exposed to 3 and 20 mM glucose. Although they were not observed in the secretions, numerous unidentified peaks were observed in islet lysates potentially pointing to their presence. Further optimization of the method is required to lower the LOD for DA and to identify the unknown peaks.

Keywords: Amino Acids, Capillary Electrophoresis, Chiral Separations

Application Code: Bioanalytical

Methodology Code: Capillary Electrophoresis
Separation of DNA has wide application in biology, medicine, human health and forensics. In most cases, the separation is based on DNA fragment length. Fragments of same length can then be sequenced or separated by sequence. Even though researchers are working on new generation sequencing techniques, DNA sequencing is still tedious and sometimes not practical for certain samples. Separation based on sequence would be satisfying as a rapid way to acquire information about the sequence diversity of same-length strands. Although several sequence-based separation methods are available, they usually require sufficient differences in conformation or in resistance to thermal or chemical denaturation and sometimes even the knowledge of target sequences. McGown group discovered that same-length fragments of single-stranded DNA differing by only one or two bases could be separated by using salt solutions in capillary zone electrophoresis (CZE). To investigate the mechanism of sequence-based separation, we characterized the charge and size (conformation) of DNA using multiple biophysical techniques, such as fluorescence anisotropy, small angle X-ray scattering, showing differences among different sequences. To further investigate how the sequence components would affect DNA’s mobility, we created a DNA library to build an empirical model of DNA mobility as a function of sequence components to shed light on the sequence-based separation.

Keywords: Bioanalytical, Capillary Electrophoresis
Application Code: Bioanalytical
Methodology Code: Capillary Electrophoresis
**Session Title**: Capillary Electrophoresis

**Abstract Title**: Reversible Addition-Fragmentation Chain Transfer Polymerization Method for Preparation of Coated Capillary and Its Application in Bottom-Up Proteomics by Using Capillary Zone Electrophoresis-Electrospray-Tandem Mass Spectrometry

**Primary Author**: Zhenbin Zhang

**University of Notre Dame**

**Co-Author(s)**: Norman Dovichi

**Date**: Monday, February 26, 2018 - Morning

**Time**: 10:25 AM

**Room**: 205C

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**Abstract Text**

**Introduction**
Capillary zone electrophoresis (CZE) is attracting increased attention for mass spectrometry-based proteomic analysis. While separation is based on the charge-to-size ratio of the analyte in CZE, the characteristics of the capillary play important roles in the electrophoretic performance. Various capillary coatings have been developed to overcome these problems. Currently, the most widely used coating method is based on free radical polymerization reactions. However, free radical scavengers can be present at trace levels. To overcome these problems, we describe an environmentally friendly coating method based on surface-confined aqueous reversible addition-fragmentation chain transfer polymerization reaction for covalently bonding polymers to capillary inner surfaces.

**Methods**
100 cm of the capillary (365 μm o.d. x 50 μm i.d., 105 cm total length) was pretreated with chain transfer agent (cyanomethyl [3-(trimethoxysilyl)propyl] trithiocarbonate) for the preparation of the coating. After that, a polymerization mixture was prepared by mixing acrylamide and 4,4'-azobis(4-cyanovarlic acid) in an acetate buffer at room temperature and stirring for about 10 min under nitrogen to form a homogeneous solution. The mixture then was introduced into the pretreated capillary and incubated at 60 °C. The coated capillary was flushed with methanol to remove residuals in the capillary and conditioned with the background electrolyte before CZE analysis.

**Preliminary Data or Plenary Speakers Abstract**
By loading 50 ng of E. coli digest, 977 protein groups and 5605 peptides were identified. The system was also applied to the analysis of 25 ng of a HeLa digest, 2158 protein groups and 10005 peptides were identified.

**Novel Aspect**
The solid phase extraction column (e.g., strong cationic exchange monolith) could be prepared at the inlet end of the capillary to further improve the identification performance of CZE-ESI-MS/MS for bottom-up proteomics analysis.

**Keywords**: Capillary Electrophoresis, Mass Spectrometry, Proteomics, Tandem Mass Spec

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

**Methodology Code**: Capillary Electrophoresis
Protein Cross-Linking Capillary Electrophoresis at High-Throughput for Assay of Protein-Protein Interactions

Investigation of protein-protein interactions (PPIs) by capillary electrophoresis (CE) has a number of advantages including low sample volume consumption, direct detection of protein complexes, and potential for high throughput. However, method development for CE of PPIs can be challenging due to the need to maintain native interactions during the separation. Protein cross-linking capillary electrophoresis (PXCE) eliminates this need by covalently cross-linking a pre-equilibrated mixture of interacting proteins prior to sieving electrophoresis. A 10 s cross-linking reaction was found to be sufficient for analysis of a variety of protein interactions. Dimeric PPIs with dissociation constants (K\[sub\]d\[/sub\]) ranging from low nM to low µM were analyzed quantitatively. For example, using PXCE heat shock protein 90 (Hsp90) was found to form a homodimer with a K\[sub\]d\[/sub\] = 2.1 ± 0.3 nM while the heterodimeric complex of heat shock protein 70 (Hsp70) interacting with heat shock organizing protein (HOP) was determined to have a K\[sub\]d\[/sub\] = 3.8 ± 0.7 µM. The method is sensitive to point mutations in the protein interaction site as well as allosteric small molecule PPI inhibitors.

The throughput of the capillary sieving separation was increased to 60 s/sample by overlapping sample separations in a high field of 1 kV/cm. At these throughputs a dissociation constant was determined in 20 min and a dose response curve was completed in 10 min. Results were found to be in good agreement with accepted methods including isothermal calorimetry (ITC) and fluorescence polarization (FP) however, the sample requirements and throughput of the PXCE method were greatly reduced in comparison to ITC and the PXCE method does not suffer from interference from peptide mimics or fluorescent artifacts possible with FP. The potential for further increasing the throughput by microchip electrophoresis will be explored.

Keywords: Capillary Electrophoresis, Lab-on-a-Chip/Microfluidics, Protein
Application Code: Bioanalytical
Methodology Code: Capillary Electrophoresis
The national backlog in sexual assault cases is estimated to be between tens of thousands to a half million untested rape kits. Current methods are time and labor intensive, requiring overnight procedures and delivering a lower than 40% success rate. The primary challenge crime labs face in analyzing these cases is the separation of purified male DNA from the mixture of primarily female DNA from gynecological swabs. Effective elution of the sample from the swab and efficient separation of intact sperm cells from epithelial and other cellular debris allow for a successful analysis of the perpetrator DNA. Capillary zone electrophoresis (CZE) is a promising tool to perform the cell separation and has three major advantages over alternative technologies: small amount of sample is consumed, which allows for replicate analyses of limited available evidence; rapid separation time compared to standard methods; and single cell detection and collection when interfaced with an automated fraction collector developed in-house.

In this work, simulated sexual assault samples are eluted from cotton swabs and the mixture is directly electrokinetically injected into a novel CZE system where intact cells and lysed cellular matrices are separated by their unique electrophoretic properties. Sample eluted from the distal end of the capillary is collected into individual wells on a microtiter plate which correspond to a CZE migration time interval. Real-time PCR amplification is used to confirm the separation and collection of purified aliquots of male DNA from spermatozoa and female DNA from epithelial cells and lysed cellular debris. A quantitative and qualitative assessment is performed of the total human and human male DNA in each well of the microtiter plate. Furthermore, the ratio of male to female DNA in each well is assessed, verifying a clean separation of the eluted mixture. The product is then prepared for a short tandem repeat (STR) analysis for forensic identification.

Keywords: Capillary Electrophoresis, Forensics, Forensic Chemistry, Separation Sciences
Application Code: Homeland Security/Forensics
Methodology Code: Capillary Electrophoresis
Predrill background studies to support hydraulic fracturing activities in the various shale plays are a common risk management strategy. Ground water analysis for methane and other light hydrocarbon gases is a critical component of these studies. No fully validated USEPA methods available yet. ASTM recently released D8028 to address many of the limitations of the previous analytical options. Other organizations are also considering development of improved methods. One company is developing water based reference materials that can be used as external proficiency test samples, analysis batch QC samples and calibration standards. Thus, impartially demonstrating method performance in the near future will be more easily accomplished.

Analytical process differences between handling of water samples and gas phase based standards can introduce a low bias. These biases arise from analyte losses and non-equilibrium conditions. Use of water based reference materials facilitates evaluation of each step in the analytical process. Switching to water phase calibration standards can significantly improve the accuracy of the final results. The stability of water based standards has been studied in several different storage scenarios. Stability ranges from a few hours to several months. Adding a representative surrogate compound at the beginning of the sample preparation process tracks the integrity of the process for every sample. Selecting the optimal difluoro-, trifluoro or tetrafluoro- ethane compound will depend on the specific hydrocarbon gases of interest, the chromatographic column and GC parameters. The optimal HFC will have similar characteristics to the hydrocarbons, but fit in a chromatographic gap between analytes.

Keywords: Environmental/ Water, Fuels\Energy\Petrochemical, Gas Chromatography, Headspace
Application Code: Environmental
Methodology Code: Gas Chromatography
Environmental - Part I

Use of TD-GC-MS for the Analysis of Semi-Volatiles Organic Compounds in Air and Materials: Applications of Automated Sample Re-Collection for the Analysis of PAH, PCB and Phthalates

Semi-volatile organic compounds (SVOCs) are organic chemicals with high temperature boiling points (240-260°C to 380-400°C). SVOCs can occur in significant abundances both in the gas phase and on surfaces, therefore they are recognised as human health concerns. In the present study, we demonstrate the excellent performance of thermal desorber coupled with GC-MS for the analysis of selected target compounds from several SVOCs chemical classes including: polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) and phthalates. Furthermore, we describe the benefits and application of sample re-collection and analysis for sample validation of analyte recovery. This innovation allows the split affluent, from primary (tube) or secondary (trap) desorption to be re-collected onto a conditioned sorbent tube. Therefore, re-collection allows a sequence of repeat analyses to be carried out on a single sample. This technique is particularly useful during method and or data validation and when repeat analysis is required. Results from primary and repeated analyses of the range of SVOCs classes used showed an excellent recovery across the entire range of compounds including the least volatile compounds in the standard mixes.

Abstract Text

Co-Authors: Ilaria Ferrante, Massimo Santoro, Natasha D. Spadafora, Nicola Watson

Keywords: Air, Environmental/Air, Gas Chromatography/Mass Spectrometry, Thermal Desorption

Application Code: Environmental

Methodology Code: Sampling and Sample Preparation
With the acceptance of Solid Phase Extraction as a viable alternative to LLE for the analysis of semi-volatile organic compounds in waste water matrices, there is a demand for reliable and proven processes for environmental labs to easily adopt. The need for fully validated protocols and robust standard operating procedures is vital for labs to be able to rapidly incorporate any new methodology with the least amount of overhead.

Using an automated SPE system, a complete data validation protocol has been developed for EPA 625.1 that can be referenced and easily incorporated by testing labs wishing to make the transition from LLE to SPE. The validation protocol comprises the initial start up of IDC and MDL runs, various real world and synthetic matrix verifications, PT data for all required analytes, and a complete reference SOP. Runs for full 1 L samples as well as low sample volumes for all matrices tested are available, as well as analyses on various Mass Spec platforms.

Specific detail is provided on dealing with real world waste water samples, particularly samples with high amounts of organic particulate matter. Direct comparisons for such matrices between LLE and SPE extracts are made, and also issues SPE solves for difficult sample matrices (emulsion formulation, water separation) are shown.

A fully automated and validated Solid Phase Extraction method is now available for EPA 625.1 waste water samples. The US EPA has approved the automated SPE method for 625.1 in August of 2017.

**Keywords:** Environmental/Water, Gas Chromatography/Mass Spectrometry, Instrumentation

**Application Code:** Environmental

**Methodology Code:** Sampling and Sample Preparation
Assessing the Occurrence of Trihalomethanes and Haloacetic Acids from Chlorinated Industrial Discharges at Sea and Their Toxicity on [i] Paracentrotus Lividus[/i]

Chlorination is also considered to be one of the most effective available treatments for the control of biofouling. However, chlorine is very reactive towards natural organic matter, ammonia and bromide present in seawater. The reactions of chlorine with these species lead to the formation of several brominated or chloro-brominated by-products, whose nature, levels and eco-toxicities are poorly documented in the literature.

This study aims at evaluating the levels and impacts of chlorinated discharges in a heavily industrialized area, located in a semi-enclosed bay in South East of France (Gulf of Fos, Mediterranean Sea). In this area, the total amount of daily releases is estimated to reach up around 6 million cubic meters of chlorine.

Two sea sampling campaigns were carried out in 2017. Water samples were collected and stored at 4°C after the addition of ascorbic acid. Samples treatment consisted of liquid-liquid extraction with MTBE. For the analysis of HAA, esterification was conducted using acidified methanol. All extracts were analyzed using GC-ECD. Additionally, individual and mixture toxicity were measured on larvae of Mediterranean Sea urchin [i] Paracentrotus lividus [/i]. The toxicity test was based on mortality, abnormal rate and stunted growth rate to characterize the developmental effect, and biochemical analysis to evaluate oxidative stress or DNA damage. Individual molecule toxicities were plotted on a dose-response curve, and toxicities on sea urchins showed whether locally adapted to the environment of the Gulf of Fos, or not, were compared. This work showed the predominance of bromoform and bromodichloroacetic acid in the industrial gulf. The toxicity of these molecules on [i] Paracentratus lividus [/i] larvae has been demonstrated for concentrations obviously higher than the environmental ones.

Acknowledgements: This work was included in the project “Fos-Sea” funded by the French Research Agency (ANR-16-CE34-0009).

Keywords: Environmental/Water, Identification, Semi-Volatiles, Trace Analysis
Application Code: Environmental
Methodology Code: Gas Chromatography
Polycyclic aromatic hydrocarbons (PAHs) are common environmental pollutants that result from incomplete combustion of carbonaceous materials. Many of the PAHs are considered to be toxic because of their carcinogenic nature and are regularly monitored in air, water and soil. Gas chromatography-mass spectrometry (GC/MS) is the most common analytical technique used for monitoring and measuring these compounds in a variety of matrices. The single quadrupole mass spectrometer has been the mainstay for this analysis for a number of years. There is an ever increasing need to detect lower levels of PAHs in a variety of matrices ranging from air and water to contaminated soil to biological cells.

In this study we present an optimized workflow for determination of PAHs achieving very low limits of detection (LOD) using the single quadrupole GC/MS. We determine PAHs in soil and optimize the GC inlet, oven, and column and MS conditions to achieve very low levels of detection. Apart from LOD and limits of quantitation (LOQ) we will also present other relevant analytical performance figures of merit like repeatability at various levels and linearity of the calibration curve. Chromatographic peak shapes for PAHs are well known for being sensitive to GC inlet and column conditions as well as MS transfer line and ion source temperature and tailing peaks shapes affect all the above mentioned analytical figures of merit. We show that our method results in excellent tailing factors even for the more difficult high boiling PAHs. Finally, robustness of the method is very important especially in a high-throughput lab which requires minimizing the downtime that results from doing inlet maintenance or cleaning the source. We show the robustness of our method and system by performing number of matrix injections and showing the number of injections the system is able to run without any unplanned downtime.

Keywords: Gas Chromatography/Mass Spectrometry, GC-MS, PAH, Trace Analysis
Application Code: Environmental
Methodology Code: Gas Chromatography/Mass Spectrometry
Here, we report our latest performance assessment results on a [micro]GC prototype, introduced at Pittcon ’17, in which all key analytical components are microfabricated from Si, including a dual-adsorbent [micro]preconcentrator/focuser ([micro]PCF), a 2.6×7 cm [micro]column chip with a 6-m long zone-heated separation [micro]channel, and an array of 4 [micro]chemiresistors ([micro]CR) with gold-thiolate-monolayer protected nanoparticle (MPN) interface films. Designed to be worn on the belt of a worker for near-real-time quantitative measurements of moderately complex mixtures of volatile organic compounds (VOC) every 5-10 min, the battery-operated prototype, termed Personal Exposure Monitoring Microsystem (PEMM), measures 20×15×9 cm, weighs ~2.1 kg, and requires only 6.6 W of average power. Building on previously reported results for a simple mixture, we have recently used the PEMM prototype to determine the components of a 21-VOC mixture spanning a 500-fold range in vapor pressure in 3.5 minutes (1 min sample; 2.5 min separation). The [micro]PCF can be operated in a split-flow injection mode to improve the resolution of early eluting analytes by [greater than] 50% compared to splitless mode. Three pairs of VOC within the mixture partially co-eluted under optimized conditions. However, the sensor array detector permitted ‘deconvolution’ of these peak pairs by chemometric methods and recognition of these (and all other) vapors by pattern recognition. Limits of detection ranged from 1-5 ng, corresponding to 80-200 ppb for a 5-mL air sample. Mock field testing preparations showed good agreement with a reference GC-FID for a 5-VOC mixture ranging [greater than] 20-fold in concentration and measured every 5 min for 1 hr.

Keywords: Gas Chromatography, Portable Instruments, Sensors, Volatile Organic Compounds
Application Code: Environmental
Methodology Code: Gas Chromatography
AIM: To demonstrate the cutting-edge performances of a miniaturized carrier-gas-free GC system, based on MEMS micromachined devices, for real-time monitoring of trace level of aromatic compounds BTEX (sub-ppb concentrations of Benzene, Toluene, Ethylbenzene, Xylene).

METHODS: GC technique is currently used to analyse outdoor air samples, monitoring environmental pollutants derived from the industrial activities and vehicular traffic. For this purpose, in this work a new miniaturized GC instrument has been developed based on MEMS technology with 1) pre-concentration column containing an innovative stationary phase, compatible with a high oxygen content in the gas-flow during de-sorption allowing the use of filtered air as carrier gas, 2) 0.5mt packed micromachined separation column with cross section of 0.64mm2 and 3) PID detector with high-sensitivity.

RESULTS: The miniaturized GC implements a purge&trap pre-concentration step using an innovative micro-cartridge, followed by thermal injection into a micro-GC column and PID detection. New long-life lamps allow maintenance-free operation periods longer than 6 months. Sensitivity depends on the duration of the pre-concentration step: with a duration of the cycle of 15 minutes, the detection limit of benzene achieved is 0.3 ppb. However, if the duration is increased up to 60 minutes, the detection limit reaches 0.1 ppb.

CONCLUSIONS: A new miniaturized, portable and sub-ppb level monitoring system for real-time environmental monitoring of aromatic compounds BTEX has been developed. The main break-through beyond state-of-the-art is the stand-alone operability of the GC system, which does not require carrier gas cylinders to perform the analytical sequence.

Keywords: Gas Chromatography, Portable Instruments, Purge and Trap, Volatile Organic Compounds
Application Code: Environmental
Methodology Code: Gas Chromatography
Multivariate Statistical Analysis of Polycyclic Aromatic Hydrocarbons Emissions and Its Alkyl and Nitro Derivatives from Diesel/biodiesel Blends

This work presents the emissions of Polycyclic Aromatics Hydrocarbons (PAHs), alkyl PAHs (APAHs), and nitro PAHs (NPAHs). The emission were measured using a Euro III engine, typical of the urban bus fleet of Brazilian cities, using different biodiesel content at different rotations. The compounds in the gas and particle phase were sampled employing XAD-2 cartridges and Teflon filters, respectively. Chemical analyzes were performed by gas chromatography with mass spectrometry detection. The results were processed using multivariate statistics using R Language. The main observation is that the emission of PAHs, A-PAHs and N-PAHs decrease with increasing biodiesel content, mainly because it is an oxygenated fuel, which promotes a better combustion. The main PAHs and derivatives have 3 to 5 aromatic rings. The results indicate a positive correlation between the PAHs, between the APAHs, with exception of 1-methyl fluorene and NPAHs except 3-nitro phenanthrene. An emission increase with engine rotation was also observed, but more pronounced for N-PAHs, probably due to the increase of NOx emissions.
Testing of fatty acid methyl esters (FAMEs) by GC is valuable in determining both an oil’s unique fingerprint as well as authenticity. As such, it is a routine method that is commonly performed, especially for testing of products with high incentive for adulteration such as olive oil. Historically, GC columns with polyethylene glycol stationary phases were popular for the analysis of simple FAMEs. Analysis of complex cis/trans and detailed FAMEs, however, is typically challenging to complete in a short time period using these columns. In this study, we present an optimized high-cyano stationary phase, Zebron ZB-FAME, designed to improve resolution of complex cis/trans FAME mixtures. Commercially available oils, including olive oil, were derivatized and analyzed using traditional phases along with the optimized ZB-FAME column. The potential for using two-dimensional GC to improve separation is also explored. The experiment revealed key differences in resolution and run time amongst the phases, as well as a high potential for time and cost savings using the optimized phase. The same method can be extended to a two dimensional gas chromatography for separation of FAMEs.

Keywords: GC, GC Columns, GC-MS
Application Code: Food Safety
Methodology Code: Gas Chromatography
Verification of the declared geographical origin of food becomes an important issue due to global challenges with substandard and falsified products. In this study, world famous brands of dry-cured ham – Iberian (Spain and Portugal), Bayonne (France), San Daniele and Parma (Italy) - were investigated with aims to evaluate the feasibility of applying multielemental and Sr isotopic compositions to identify their geographic origin. The original analytical data for thirty-four trace and ultra-trace elements determined by high performant quadrupole inductively coupled plasma mass spectrometry (Q-ICP-MS) is presented. The variation of elemental composition considers as promising constituents to be used for distinguishing of Bayonne ham from hams other origin. Strontium isotope ratio 87Sr/86Sr of dry-cured hams determined by multicollector inductively coupled plasma mass spectrometry (MC-ICP-MS), consider an additional distinctive parameter which presents remarkable improvement for Bayonne ham discrimination. The authors bring attention to the important fact that food processing features can be also traced by isotope analysis and became an important signature in proving of food authenticity and safety.

**Keywords:** Food Identification, Food Safety, ICP-MS, Isotope Ratio MS

**Application Code:** Food Safety

**Methodology Code:** Process Analytical Techniques
Ion Chromatography (IC) is a workhorse for the separation of all kinds of polar or charged analytes. Typically, separated species are detected using suppressed conductivity or UV absorbance, in some cases after post column derivatization. For some species though, especially when they represent different oxidation states of trace elements, or contain trace elements as part of the chemical structure, inductively coupled plasma mass spectrometry (ICP-MS) can be a powerful alternative to existing detectors, enabling lower limits of detection and wider dynamic ranges.

For some analyses, such as arsenic in fruit juices or bromate speciation in drinking waters, the combination of IC as a separation technique and ICP-MS as an element selective detection system has found its way into regulatory definitions, for example method 321.8 published by the Environmental Protection Agency (EPA).

Another application for which IC-ICP-MS is ideally suited is the speciation of hexavalent chromium (Cr (VI)) at low levels in drinking waters. Here, the inherent absence of metal containing parts in an IC system is an obvious advantage over other approaches. A second advantage is the ability for simple and quick separations utilizing dedicated column chemistries, which allow elution of both Cr species (toxic Cr (VI) and essential Cr (III)) using dilute nitric acid in less than three minutes.

This presentation will highlight dedicated applications of IC-ICP-MS for speciation analysis and will show that this unique combination has numerous advantages for modern laboratories dealing with speciation analysis in different market segments, such as food analysis or industrial process control.
Inorganic cation determinations are important for food product labeling and to ensure product quality. Additionally, early indicators of food spoilage can be assessed by monitoring for the appearance of alkylamines. In other industries, such as the petroleum industry, alkanolamines (monoethanolamine, diethanolamine, and methylmonoethanolamine, and methyldiethanolamine) are used routinely to prevent corrosion during transportation to the refinery or to remove sour gases during the refining process. Processing plants require accurate analytical methods to characterize and determine the next refining steps needed for oil and gas products received from various oil, gas, and fracking wells. Ion chromatography coupled to a single quadrupole mass spectrometer (IC-MS) is an ideal and economical way to determine and confirm cations and amines. Here we demonstrate cation, alkylamine, and alkanolamine determinations in spoiled beverages and amine solutions, respectively, by cation-exchange separation followed by suppressed conductivity and mass spectrometry detections in a serial configuration. Cations, alkylamines, and alkanolamines were determined in full scan from m/z 18 to 500 and individual SIMs as bare ions and when further sensitivity is needed, as their hydrated adducts. Unlike earlier IC-MS methods for cation determinations, the new single quadrupole MS used in this required no organic solvent for desolvation. Limit of Detection were single digit µg/L for most analytes. The experiments showed that typically ethylamine concentrations were prominent in spoiled juice and tea samples. In another sample, the green tea sample exhibiting mold for 3 days had ~2x higher monoethanolamine and ethylamine than the freshly prepared green tea sample.
Coffee is one of the most popular drinks in the world and is a major world commodity. Over 7 million metric tons of coffee is produced annually. Two species of coffee beans are of primary importance in the commercial coffee industry: Coffea canephora (i.e. 'robusta') and C. arabica. Roasted coffee beans can contain well over a thousand different compounds. In most cases the most important of those chemicals are the flavor compounds (i.e. chlorogenic acids, polyphenols, terpenes, etc.) and caffeine. Other compounds such as methyl cafestol and Homostachydrine can be important in fingerprinting the authenticity and adulteration of coffee and coffee species.

Chemical profiles of coffee around targeted groups of chemical markers in coffee were created to determine: caffeine content (and decaffeination by-products), coffee or species authenticity, and adulteration and safety of coffee using three different analytical techniques: LC-MS, FTIR and UV slope spectroscopy.

The goal of the study was to determine if popular coffee products met the expected claims of the providers as to identity, flavor and caffeine content. Brewed regular roast and decaffeinated coffee was purchased from multiple chain fast food restaurants, coffee purveyors and convenience stores. Samples of regular home brewed and pod brewed regular coffee were also tested for caffeine content, authenticity and flavor markers. Chemical profiling of some coffee samples showed misrepresentation of coffee species in some brewed coffees as well as the presence of higher than expected levels of caffeine in some samples. Also present were chemicals which indicated signs of either degraded coffee flavor or potential adulteration.

Keywords: Beverage, Food Safety, FTIR, Liquid Chromatography/Mass Spectroscopy
Application Code: Food Safety
Methodology Code: Liquid Chromatography/Mass Spectrometry
Polar pesticides while actually herbicides (glyphosate, glufosinate, N-Acetyl glufosinate, ethephon, fosetyl-Al, and maleic hydrazine) and their metabolites (N-acetyl AMPA, AMPA, HEPA, 3-MMPA) are increasingly a public and a regulatory concern in food. Ion chromatography (IC), designed for the determination of ionic compounds in a variety of samples, including food, is better suited for separating polar pesticides than other chromatography methods. Additionally IC is a direct determination method unlike GC which requires labor intensive and costly derivatization. To provide accurate reporting when testing herbicides and pesticides, molecular identification and confirmation are critical. High resolution accurate mass mass spectrometry (HRAM MS) provides sensitivity to part per billion or part per trillion due to the low chemical noise provided by the IC separation and the high resolving capabilities of the Thermo Scientific Orbitrap MS. Disinfection byproducts and perchlorate contaminations are also contamination concerns in food, but typically require separate determinations. Here we combine the determinations of polar pesticides, perchlorate, and disinfection byproducts in diluted fruit juices and beer samples into one method. All analytes are separated by IC on a 2 x 150 mm anion-exchange column within a 15 min run and detected serially with suppressed conductivity and HRAM MS in full scan from 60–750 m/z and in SIM mode.

Keywords: Beverage, Ion Chromatography, Liquid Chromatography/Mass Spectroscopy, Mass Spectrometry
Application Code: Food Safety
Methodology Code: Liquid Chromatography/Mass Spectrometry
Emerging and fugitive contaminants (EFCs) generated by anthropogenic activities have caused a fugitive legacy threat to the quality and quantity of food and water, which are closely linked through plants. Rapid freeze-thaw/centrifugation extraction followed by high performance liquid chromatography - tandem mass spectrometry (HPLC-MS/MS) methods were developed in this study for determination of twelve EFCs, including Estriol, Codeine, Oxazepam, 2,4-DNT, RDX, Acetaminophen, Bisphenol-A, Triclosan, Caffeine, Carbamazepine, Lincomycin, DEET, in different kinds of plant, i.e. corn (Zea mays), tomato (Solanum lycopersicum) and wheat (Triticum spp). The method centrifuges the sap out of the plant tissue through a molecular sieve membrane filter directly in the centrifugation tube to remove macromolecules and particulates from the sap. The sap solution can then be analyzed directly by HPLC-MS/MS methods, one by positive ionization and one by negative ionization. Method limits of detection (LOD) ranged from 0.01 \( \mu g.L^{-1} \) to 2 \( \mu g.L^{-1} \) and limits of quantification (LOQ) ranged from 0.02 \( \mu g.L^{-1} \) to 5 \( \mu g.L^{-1} \) in sap. The recoveries of spiked analytes in three kinds of plant ranged from 83.6% to 107.2%. These methods offer a very rapid approach to determine the EFCs concentration in agriculture crops, therefore their uptakes and distribution could be investigated. Such understanding will allow preemptive mitigation of food and water contamination as well as targeted sampling of specific compounds.

This study was supported by National Science Foundation (NSF).
A panel of representative polar pesticides, including aminomethylphosphonic acid (AMPA), chlorate, ethephon, fosetyl aluminium, glufosinate, glyphosate, maleic hydrazide and phosphonic acid, were targeted in a single liquid chromatographic tandem quadrupole mass spectrometry method. Extracts of tomato juice, apple juice, & beer were prepared in accordance with the Quick Polar Pesticides (QuPPe V9.2) extraction method. Chromatographic separation was achieved on mixed mode / hydrophilic interaction liquid chromatography (HILIC), applying a mobile phase gradient. Targeted multi-reaction monitoring (MRM) methods were used, with at least two transitions per compound and +/- 30% for ion ratios, to quantify and confirm analyte detection.

Chromatographic performance was evaluated in accordance with SANTE guideline document 11954/2015. Following evaluation of a selection of mixed mode LC columns, certain limitations were determined for the challenging analysis of these highly polar pesticides. This evaluation was for an LC-MS/MS method for the direct analysis of non-derivatized anionic pesticides. Retention of all analytes was greater than two times the void volume, while the retention time tolerance for each analyte was within ±0.1 minute of the corresponding matrix matched standard. Excellent sensitivity was achieved for all analytes in matrix below 0.001 mg/kg (except for ethephon in tomato juice at 0.0025 mg/kg). Residues of chlorate, maleic hydrazide and phosphonic acid were detected in samples.

Overall method performance, in the absence of isotopically labeled internal standard, was evaluated by assessing linearity, accuracy and sensitivity.

Keywords: Food Contaminants, Liquid Chromatography/Mass Spectroscopy, Pesticides, Tandem Mass Spec
Application Code: Food Safety
Methodology Code: Liquid Chromatography/Mass Spectrometry
Using active learning techniques has been shown to be an effective way of achieving course objectives in the chemistry curriculum. The implementation of some of these techniques can be achieved with new technology developments available to most faculty members. In this trial study, exam review in a second-year analytical chemistry course was facilitated through the use of a free, online response system that utilizes mobile phones and laptops as “clicker-like” devices. In addition to instantly determining students’ comprehension of content areas, certain versions of these systems enable competitive scoring based on speed and accuracy that can be displayed to the class throughout the review session. Here, the use of one such system (Kahoot!) was tested for exam review related to the six areas covered by the American Chemical Society (ACS) Analytical Chemistry Comprehensive Exam (solution chemistry, experimental methods, acid-base chemistry, spectroscopy, separations, and electrochemistry). Advantages and limitations of its use for this content area were determined and compared to functions available in other web-based response systems. To increase the use of this system in similar courses at other institutions, descriptive metadata to find content quizzes at an appropriate level for the ACS Comprehensive Exam was implemented. Due to the lack of formal review materials that are available through the ACS, these shareable quizzes provide a much-needed source of example questions for courses that use this exam. In general, students found Kahoot! activities to be an interesting and fun way to review this material, indicating continued use is warranted, although the true effect on test scores has yet to be determined.
Innovations in Teaching

Gas Chromatography of Electronic Cigarettes: A Demonstration of the Importance of Internal Standards in Quantitative Analysis

A solid understanding of the appropriate use of internal standards and standard addition in Quantitative Analysis is imperative. Unfortunately, many students complete the course without the ability to differentiate between these methods or to apply them appropriately. To increase student understanding of these techniques, a multi-week lab method was designed that incrementally and inductively demonstrated the rationale behind each technique. Using gas chromatography with a non-selective flame ionization detector, students were able to quantitate nicotine in the solution utilized in electronic cigarettes. While the traditional model utilizes either standard addition or internal standards, this method had students performing both simultaneously. This allowed for the students to compare and contrast both methods, and facilitated a better understanding of the usefulness of each in the lab, as well as when to use them. Additionally, because injection volume is a major source of error in manual GC analysis, student work without an internal standard is often inundated with error. To account for this, students prepared samples using a traditional standard addition, but also incorporated an internal standard. By performing calculations with and without the internal standard, students could see the advantages of using an internal standard and better understand its importance in the field. The resulting data were analyzed algebraically and graphically, and GC-MS was used to corroborate the data. This work demonstrates for the first time how both methods can be used simultaneously to teach students the importance of sensitivity, selectivity, matrix effects, error, and the importance of methodology in Quantitative Analysis.

Abstract Text

Keywords: Calibration, Gas Chromatography/Mass Spectrometry, Standards, Teaching/Education
Application Code: Consumer Products
Methodology Code: Education/Teaching
We live in a data-intensive society. But are we truly training our children to be ready for a genomics- and data-rich future?

PlayDNA (www.goplaydna.com) teaches data science to middle and high school students using an innovative STEM curriculum that involves hands-on generation of big data through portable DNA sequencers, inside the classroom.

Data science and coding are topics that are challenging to teach to students between 12 and 18 years old. Students do not immediately see the relevance of these skills. PlayDNA enables big data generation and analysis in the classroom: instead of being confronted with static data-frames the students build and analyze data-frames themselves through biological questions and DNA sequencing. The curriculum takes students on a journey where students convert DNA bases to computer bits and interpret the data. Through deep understanding of the question, ownership over the data and understanding of how an analysis is built, PlayDNA aims to enthuse students to learn how to code.

PlayDNA makes the next generation data- and genomics-literate, so everyone can make educated choices in their future endeavors.

Keywords: Data Analysis, Education, Genomics
Application Code: Genomics, Proteomics and Other ‘Omics
Methodology Code: Education/Teaching
Innovations in Teaching

Enabling Low-Cost Data Acquisition with Open-Source Microcontrollers

Most analytical instrumentation signals are converted to voltages that are then recorded by data acquisition systems (DAQs). In both research and teaching labs, this is often achieved through the use of USB-enabled DAQs that contain a 12- or 14-bit analog-to-digital converter (ADC). In addition to this hardware, software control for data recording is also needed, which can add higher costs to each system and is especially cost-prohibitive when several units are required for multiple instruments. Over the past decade, open-source microcontrollers implementing easy-to-write software languages for simple hardware control have become increasingly popular, leading to a so-called “maker movement” among scientists and engineers. A number of analytical tools, including pH meters, photometers, and automatic titrators, have been integrated with these microcontrollers to reduce cost and increase user control. Here, an Arduino Uno and a 16-bit ADC integrated circuit were combined to create an open-source DAQ that has higher resolution than many commercial DAQs at a fraction of the cost. The system includes Python hardware that can be easily downloaded and installed on most computers with only a USB connection required for data acquisition. Compared to the standard acquisition systems used in gas chromatography and capillary electrophoresis instrumentation, the new DAQ provided identical data output, demonstrating its potential to replace these systems for similar uses. Further miniaturization of the system by replacing the computer required for Arduino control with a Raspberry Pi platform was also explored.

Keywords: Data Analysis, Instrumentation, Separation Sciences, Software
Application Code: General Interest
Methodology Code: Education/Teaching
**Abstract Text**

Here we present our recent development, the atomic force microscopy (AFM) technique for quantitative analysis of mechanical properties of soft materials at the nanoscale. This add-on to the existing AFMs is capable of highly accurate measuring dynamic mechanical analysis (DMA) of material properties. This mode (suggested name FT-NanoDMA) is a combination of three different methods: the quantitative dynamic mechanical spectroscopy (DMS), AFM indentation, and Fourier-transform spectroscopy. This mode is fast and sensitive enough to allow DMS imaging of nanointerfaces and single biological cells. Compared to the existing state-of-the-art nanoindentation, FT-NanoDMA demonstrates ~ 100x improvements in both spatial (down to 10nm) and temporal resolution (down to 0.7 sec/pixel). We demonstrate the technique on known polymers, biological cells and polymers blends.

**Keywords:** Atomic Force Microscopy (AFM), Materials Characterization, Method Development, Polymers & Plas

**Application Code:** Polymers and Plastics

**Methodology Code:** Surface Analysis/Imaging
The hydroxyl number of polyols, an important precursor in polyurethane production, must be measured to ensure that the desired number of hydroxyl groups are available for reaction with isocyanates. This analysis is typically accomplished via potentiometric titration according to ASTM D 4274-88. The reaction typically consumes both acetic anhydride and pyridine. Near-infrared (NIR) spectroscopy can perform this same analysis without the use of reagents and requiring no sample preparation. In this poster, we demonstrate the efficacy of an NIR model we have developed that can be transferred from instrument to instrument and is applicable to nearly all polyols over a wide hydroxyl range.
**Abstract Text**

Evolved gas analysis improves the value of TGA data by allowing the identification of the species evolved during decomposition. FTIR is particularly advantageous when organic molecules and IR active gases are being analyzed from the TGA sample. This lecture will introduce an innovatively designed TGA-FTIR and STA-FTIR (STA = Simultaneous DSC-TGA) for evolved gas analysis. The unique coupling system has the FTIR now mounted directly above the sample cell, eliminating the need for a long transfer line. The system allows immediate response from the FTIR when the sample loses mass. The lack of the transfer line allows for analysis of gas species that would ordinarily condense in a standard heated transfer line in addition to dramatically reducing bench space normally required for TGA-FTIR.

**Keywords:** DSC, FTIR, Polymers & Plastics, Thermal Analysis

**Application Code:** Polymers and Plastics

**Methodology Code:** Thermal Analysis
The visual microfluidic rheometer, FLUIDICAMRHEO, offers a way to quickly determine average molecular weight by using viscosity measurements. The co-flow microfluidic system uses a small sample volume and can be used for a wide range of viscosities over a very wide range of shear rates (up to 10^5 s^-1). FLUIDICAMRHEO is particularly sensitive for low viscosity solutions which makes it an ideal method for accurate molecular weight determination. The user-friendly design of the instrument and software means that the method is suitable for fast quality control and routine testing without an expert operator.

Visual Microfluidic Rheometer Technique

FLUIDICAMRHEO uses a co-flow microfluidic principle to measure viscosity. The sample and a reference solution are simultaneously introduced into the microfluidic channel (typically 2.2mm X 150µm) with controlled flow rates. This results in a laminar flow where the interface position between sample and reference relates the viscosity ratio and flow rates. Images acquired during the measurement allow the software to calculate the position of the interface and directly plot an interactive flow curve.

Average Molecular Weight Determination

FLUIDICAMRHEO can be used to measure the viscosity of polymer solutions to determine the intrinsic viscosity which is related to molecular weight by the Mark-Houwink equation. The example shown in figure 2 is a hydroxyethyl cellulose polymer. The polymer is fully dissolved in a chosen solvent at a concentration inferior to its C^*. Multiple concentrations can be made up to produce a Huggins-Kraemer plot to determine the intrinsic viscosity graphically, see figure 2. Alternatively, the Solomon-Ciuta equation using a single concentration for an even faster determination.

Keywords: Lab-on-a-Chip/Microfluidics, Materials Characterization
Application Code: Polymers and Plastics
Methodology Code: Microfluidics/Lab-on-a-Chip
Phthalates have been used as additives in plastic for many years to make them more flexible. Several phthalates are regulated on a global scale and several analytical techniques can be used for phthalates analysis. Thermal desorption (TD)-GC/MS is one of the easiest and most accurate methods for phthalate analysis.

Recently manufactures have switched formulations to include unregulated phthalates. Generally, phthalates are identified using both retention time and MS spectra. However, some unregulated phthalates have similar retention times and MS spectra, compared to regulated phthalates. We discuss quantitative analysis of regulated phthalates in a sample with high concentrations of unregulated phthalate(s).
The properties of polyethylene (PE) can be changed by the proper choice of additives. These additives sometimes contain a halogen or sulfur. It is important to know the amounts of these elements when a plastic product, such as one produced from PE, is either disposed of or recycled. This presentation describes a method to determine the chlorine, bromine, and sulfur contents of PE products. The PE sample was thermally decomposed in an argon atmosphere and then combusted in an oxygen atmosphere by an automated combustion instrument. The resulting gas was trapped in an absorbing solution where chloride, bromide, and sulfate represent the chlorine, bromine, and sulfur in the sample. A portion of the absorbing solution was automatically transferred to an ion chromatography (IC) system to measure the chloride, bromide, and sulfate. The IC system prepared its own eluent (mobile phase) by electrolytic eluent generation thereby providing additional method automation. Sample analysis time was 30 min, with 10 min required for the IC separation. After the first sample is combusted a second sample can be prepared while the IC separation is in progress. We developed the method, evaluated its accuracy, and regularly checked its performance with European Reference Material EC680, a PE with low level trace elements. We applied the method to measuring the chlorine, bromine, and sulfur contents of a lab wash bottle, transfer pipette, “bubble wrap”, and plastic wrap.
Polymers - Measurement of Properties and Analyses

Characterization and Quantification of Cosmetic/Industrial Polyquaternary Species via Polyion-Sensitive Membrane Electrodes

Polymeric quaternary ammonium salts (polyquaterniums (PQs)) are extensively used in cosmetics and water treatment processes. Furthermore, some PQs are used in shampoos and hair conditioners. To quantify and characterize PQs, polyion-sensitive ion-selective electrodes (ISEs) can be used. Such devices were developed originally to detect heparin and protamine. There are two variations of polyion sensors: single-use and reversible (pulstrode) sensors. Single-use sensors require a new membrane be used for each polyion detection experiment. Conversely, pulstrode sensors can be reused. Pulstrode sensors are also universal polyion sensors, as they respond to polyanions and polycations.

One detection approach using either sensor variation focuses on recording direct EMF responses to polyions as the sensor is subjected to a solution containing the target polyion. Another approach is based on titrating samples with oppositely charged polyions and detecting titrant excesses with the sensor. Herein, these polyion detection methods are employed to quantitate and characterize the chemical properties of various PQs (e.g., relative diffusion coefficients in plasticized poly(vinyl chloride) membranes and relative charge densities). Single-use sensors employed for the quantification of PQ-2, PQ-6, PQ-10, and poly(2-methacyloxyethyltrimethylammonium) chloride (PMETAC) will be shown to exhibit limits of detection (LODs) of 7.7, 6.9, 21.5, and 9.0 µg/mL, respectively. Quantification of the same PQs via pulstrode sensors will be shown to exhibit LODs of 8.0, 7.9, 35.5, and 10.3 µg/mL, respectively. We will also summarize efforts to develop potentiometric polyion detection methods to quantitate various PQs using flow-through devices such as flow-injection analysis systems and microfluidic devices.

Keywords: Cosmetic, Sensors, Titration, Water

Application Code: Polymers and Plastics

Methodology Code: Electrochemistry
Among the ways of finding explosive devices, vapor detection is the most attractive since it allows stand-off and remote sensing. Unfortunately, many high explosives, including the nitroaromatics, nitramines and nitrate esters, are very difficult to detect from their vapors because they have extremely low volatilities. This is the greatest single factor that limits the performance of vapor-based detectors. One solution is to increase the effective concentration of the vapor delivered to the sensor with a pre-concentrator: a device that collects the vapors over a set period of time before releasing them in a brief pulse. In this project, we tested 3 types of sorbing phases for the fabrication of the pre-concentrator.

The sorbing phase of the pre-concentrator device is fabricated from polydimethylsiloxane (PDMS) and from its blend and copolymer with divinylbenzene (DVB) using sol gel method and dip coating. It was found that independent of the type, all sorbing phases were able to sorb an amount of nitro-based explosive analyte. However, for sorbing films of similar thickness, those formed from the PDMS copolymerized with DVB sorbed the greatest amount of analyte and had the best retention capability. Atomic Force Microscopy (AFM) suggested that PDMS copolymerized with DVB film had the highest surface area attributed to spheroid structures of the polymer film. Meanwhile, enhanced retention of the analyte in the film is due to the interaction of nitro-based analytes to the benzene moiety in the film. Finally, the best pre-concentrator film was determined to be able to increase the available analyte for vapor detection by up to 3 orders of magnitude. This enhancement of analyte concentration through pre-concentration will significantly improve the response in an explosive vapor detector.

Keywords: Polymers & Plastics, Sensors, SPME, Trace Analysis
Application Code: Polymers and Plastics
Methodology Code: Physical Measurements
Aromatic amines (AA) are present in tobacco smoke and are known carcinogens. AAs can be measured in human urine as tobacco exposure biomarkers. Monitoring these AAs in large population studies such as the National Health and Nutrition Examination Survey (NHANES) requires an efficient and streamlined sample data flow that could track a large number of samples and handles the corresponding data generated. Our laboratory developed an automated method to monitor AAs in human urine. A streamline sample data flow was created which enables sample tracking from receiving to final result reporting with minimal human intervention, further diminishing human error in the sample preparation and data processing.

The sample data flow for AA assay has six distinct sections. Once received, all urine samples are logged in STARLIMS, a laboratory information management system. The Hamilton STARTM liquid handling workstation greatly reduces prep time of sample cleanup by automating repetitive tasks. After sample clean up, the unknown samples are analyzed on a GC-MS/MS. The acquired GC/MS raw files are uploaded to Indigo ASCENT for data review and quantitation. After reviewing, certified Indigo output files are uploaded to Repeat Manager (RM). RM, a custom made program using Microsoft Access, serves as a data repository and a data manager. RM is a powerful tool for tracking results and managing repeats in case of individual samples that failed established quality assurance (QA) rules. A feature built into RM is the option to build repeat runs based on a variety of options: analyte, concentration range, blank failure, QC failure. Results that pass QA rules and quality control are exported from RM and uploaded to STARLIMS for the final evaluation and to generate the final report for the current study.

This sample data flow has been tested, validated, and is currently applied in our bio-monitoring of AAs in the US non-institutionalized population NHANES 2013-2014 cycle.

Keywords: Data Analysis, Environmental/Biological Samples, Quality Control, Sample & Data Management
Application Code: Quality/QA/QC
Methodology Code: Data Analysis and Manipulation
Impurities or degradants are unwanted components which may come from the processes of organic synthesis, formulation, and storage of pharmaceuticals or other incoming materials. Identification of these impurities is of great importance for producers, regulatory agencies, and consumers. A critical step to identify these impurities, is to determine their elemental compositions. While this can typically be achieved through higher resolution MS systems such as TOF, qTOF, Orbitrap or FT ICR MS, it is more economical and practical if it could also be accomplished on a more common and cost-effective MS System such as single quadrupole LC/MS.

Due to the unit mass resolution available on a single quadrupole LC/MS system, it typically does not result in enough mass measurement accuracy for elemental composition determination. Upon careful examination of the system’s reproducibility, however, it was found that these systems are stable with less than 0.00x amu mass shift over a few hours, indicating that a properly calibrated system may be able to achieve 0.00x mass accuracy. Through such a novel calibration process, another important metric, called spectral accuracy, could also be enhanced significantly to allow for exact and quantitative mass spectral comparison between the calibrated mass spectrum isotope profile and the theoretically calculated true mass spectrum, enabling elemental composition determination of unknown ions.

With the API simvastatin known and used as an internal calibration standard, most of the impurities of the drug were correctly identified as the top 1st or 2nd hit with excellent mass accuracy and spectral accuracy. These results demonstrate that high enough mass accuracy combined with high spectral accuracy can be achieved on a conventional single quadrupole LC/MS instrument to enable elemental composition for impurity and degradant identification from pharmaceutical, chemical or fine chemical processes.

**Keywords:** Contamination, Liquid Chromatography/Mass Spectroscopy, Pharmaceutical

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

**Methodology Code:** Liquid Chromatography/Mass Spectrometry
## Session # 230  Abstract # 230-3

### Session Title
Quality/QA/QC

### Abstract Title
Metabolomic Biomarkers for Human Plasma Quality by UHPLC-HRMS

### Primary Author
Casey A. Chamberlain
University of Florida

### Co-Author(s)
Chris Beecher, Timothy J. Garrett

### Abstract Text

**Introduction:** The degradation of plasma metabolites is highly-important from both a clinical and research perspective to ensure accurate and thorough analysis of diagnostic and experimental assays; however, little work has been done to fully understand it. Identifying the degradation products of plasma and other biologically relevant samples is critical as these compounds could serve as biomarkers for sample age and quality. This study seeks to identify changes in the metabolic profile of human plasma resulting from exposure to common storage conditions (4°C and room temperature) for 0-8 hours. This work employs Isotopic Ratio Outlier Analysis (IROA) using a 95% 13C-labeled yeast extract for universal internal standardization.

**Methods:** Pooled human plasma was aliquoted and stored at -80°C. At each time point, twelve aliquots were pulled from the freezer and set away from light either at room temperature or 4°C. Samples were frozen at -80°C once conditioning was completed. All samples within each series were thawed and processed simultaneously using our standard 8:1:1 acetonitrile:methanol:acetone metabolite extraction protocol. Plasma extracts were reconstituted in water for analysis. In parallel, a set of samples from each temperature series was reconstituted in a 95% IROA-labeled yeast extract solution serving as a broad-scale injection standard. The IROA method allows for standardization of hundreds of features simultaneously by providing a signature spectral pattern for all features of biological origin due to the unique 95% labeling of the yeast extract.

**Novel Aspect:** This work utilizes an IROA-labeled yeast extract universal internal standard to identify potential biomarkers for human plasma age and quality.

### Keywords:
- Mass Spectrometry, Metabolomics, Metabonomics, Plasma, Quality Control

### Application Code:
- Quality/QA/QC

### Methodology Code:
- Liquid Chromatography/Mass Spectrometry
Shortly after the PC was introduced, digitized databases and search software became available to identify materials characterized by spectral techniques like FTIR, MS, and XRF or chromatographic separation like GC. Until recently, however, no such intelligent, auto-searchable and user-scalable database for thermal analysis existed. Identifying unknown polymers based on their DSC and TGA profiles was still a manual, old-fashioned, knowledge-intensive process. This presentation will delve deeper into explaining a unique new thermal analysis databasing software known as IDENTIFY and apply it to the DSC and TGA traces of a leading kitchen foam scrubber featured heavily on TV, yet of "secret" composition in order to determine its polymer makeup.

The IDENTIFY software system uses an approach very similar to that employed by modern image recognition software for precisely identifying persons or objects. IDENTIFY can search through hundreds of database entries and, within seconds, isolate DSC and TGA measurement curves most similar to the unknown curves, even identifying the material by trade-name and manufacturer. This new technique also works for TMA (thermomechanical analysis) data.

Keywords: DSC, Material Science, Quality Control, Thermal Analysis
Application Code: Quality/QA/QC
Methodology Code: Thermal Analysis
The introduction of exogenous molecules into intact cells is a key steps in several bioanalytical methods. For example, peptide substrate reporters are used to report on enzyme activity in cells, but these molecules must first cross the lipid bilayer. In this work, we compare three methods of peptide substrate reporter loading: electroporation, pinocytosis, and myristoylation. Using fluorescence measurements and capillary electrophoresis, we have characterized the efficiency of peptide loading, cell-to-cell variation, peptide localization, and metabolism and phosphorylation of a reporter for protein kinase B (PKB) as a function of loading method. We find notable differences in each of these metrics between loading methods. Myristoylation was most effective in transporting the peptide across the membrane, while electroporation produced the most uniform cytoplasmic loading. In all cases, loading was heterogeneous between individual cells. These findings demonstrate that bioanalytical research requires careful evaluation of the methods used to load molecular tools into cells and offer some information to guide experimental design choices.

### Keywords:
- Bioanalytical
- Capillary Electrophoresis
- Enzyme Assays
- Peptides

### Application Code:
- Bioanalytical

### Methodology Code:
- Capillary Electrophoresis
The thrombin-binding 15 and 29mer DNA aptamers are well characterized and serve as benchmarks when developing analytical techniques. However, published values of dissociation constants for these aptamer-protein complexes vary widely, with values ranging from 25 nM to 450 nM in the case of the 15mer and 0.5 nM to 240 nM for the 29mer. The reported role of Mg2+ ions in binding is also inconsistent, with some investigators claiming increased binding in the presence of Mg2+ while others claim no effect. Finally, in affinity probe capillary electrophoresis (APCE) experiments, voltage-dependent heating of the non-cooled capillary inlet (NCI) and the subsequent dissociation of aptamer-protein complex is a source of systematic error that has not yet been validated on a robust model system like thrombin and its aptamers. Taken together, these differences indicate the ranging effects of experimental conditions on apparent affinity, motivating us to systematically explore the effects of buffer composition and instrumental technique on the thrombin model systems. We have determined binding constants using two orthogonal techniques: Fluorescence Anisotropy (FA) and APCE to observe effects of instrumentation on affinity. FA experiments were also conducted in five different buffer environments. FA experiments tended to give higher dissociation constants than APCE, as did APCE when including the NCI during separation. Experiments from FA in different buffer systems reveal little effect of buffer on apparent affinity. The dissociation constants determined in our experiments fall within the literature range, but differ depending on analytical technique.

Keywords: Bioanalytical, Capillary Electrophoresis, Fluorescence, Nucleic Acids
Application Code: Bioanalytical
Methodology Code: Capillary Electrophoresis
Affinity interactions between macromolecules form the basis for a large range of analytical application, such as diagnostics, drug development and labeling. In order to quantify the strength of an affinity interaction a stability constant, such as the dissociation constant ($K_{D}$), is often used. Measuring a $K_{D}$ requires both 1) a robust affinity assay and 2) appropriate mathematical models to analyze the affinity data. Many innovative affinity assays are available however comparatively few mathematical models have been developed for analyzing affinity data. Even less treatment has been devoted to how models compare to each other. The thrombin aptamers are a widely studied model system for which a large range of $K_{D}$ values have been reported. Differences in affinity assay and buffer have often been cited as the source of variation in $K_{D}$ values. However, the mathematical model used to analyze data has not yet been examined as a source of variation. Using affinity probe capillary electrophoresis (APCE) affinity data was collected for the thrombin aptamers. Several popular models were then applied to the APCE data using non-linear least squares and the resulting $K_{D}$ values and standard deviations compared. Out of the available model we found that the Hill equation with a complex of 1 aptamer to 2 protein molecules provided the best fit. This result is supported by the literature as well as electropherogram data and suggests the model fit can be used to identify the appropriate model for data analysis.

Keywords: Bioanalytical, Capillary Electrophoresis, Data Analysis, Nucleic Acids
Application Code: Bioanalytical
Methodology Code: Capillary Electrophoresis
Bioanalytical - Capillary Electrophoresis

Isolating Aptamers for Cancer-Linked Glycosylations of Immunoprecipitated Thrombospondin-1 Using Capillary Electrophoresis Selection - FLASH PRESENTATION

Proteins undergo a wide range of post-translational modifications (PTMs), affecting both their structure and function. There is increasing evidence that some of these modifications can be correlated with developing diseases. For example, thrombospondin-1 (TSP-1) has been demonstrated to exhibit a significant alteration in its glycosylation state in early stages of endometrioid ovarian cancer, including the addition of a core fucosylation. We have utilized CE-SELEX (capillary electrophoresis-systematic evolution of ligands by exponential enrichment) to identify aptamers that are able to distinguish between cancerous and non-cancerous glycoforms of TSP-1. This strategy has been shown to develop aptamers with moderate-to-high affinity in six or fewer rounds of selection. TSP-1 was isolated from both cancerous and non-cancerous cell lines via immunoprecipitation and used as the target analyte. Alternating positive- and counter-selection rounds versus the cancerous and non-cancerous proteins, respectively, yielded aptamer sequences with both high affinity and selectivity for the cancerous glycoforms. This work will lead to the development of aptamer arrays as diagnostic tools for developing disease.

Abstract Text

Keywords: Bioanalytical, Capillary Electrophoresis, Laser, Protein
Application Code: Bioanalytical
Methodology Code: Capillary Electrophoresis
Inorganic phosphate has an important role in both environmental processes as well as human health. In terms of human health, determination of inorganic phosphate in human urine allows for diagnosis of various ailments including bone, kidney, or glandular diseases. Inorganic phosphate is also an essential nutrient for plants and is commonly found in fertilizers to maintain the quality of soil and promote plant growth. From the soil, it can leach into bodies of water over time and can cause algae outbreaks that can be harmful to aquatic life.

Herein, the use of a Fluorescence Resonance Energy Transfer (FRET) based phosphate sensor is described. The fluorescent donor chosen for this study is a terbium-ethylenediaminetetraacetic acid complex (Tb-EDTA), while the acceptor is gold nanoparticles (AuNPs) capped with cetyltrimethylammonium bromide (CTAB).

Inorganic phosphate determination is achieved by mixing filtered water or filtered urine sample with a sensing solution of Tb-EDTA and AuNPs-CTAB. Volumes as low as 0.5 – 1 mL of sample can be used for analysis. The strong affinity between inorganic phosphate ions and CTAB molecules allows for the phosphate ions to act as an anion bridge between AuNPs, resulting in aggregation and precipitation of the AuNPs out of solution. Upon precipitation, the AuNPs can no longer act as the FRET acceptor, thus restoring the photoluminescence observed from Tb-EDTA. The intensity of the luminescence signal has a linear correlation to the concentration of phosphate in the sample. The sensor has a limit of detection of 83 parts-per-billion (ng.mL-1) of phosphate, which is well suited for the concentration range of phosphate found in urine and water samples.
This study describes a bottom-up assembly route for monodisperse carbon dots (CDs) into different size of the CD aggregates. The highly monodisperse CDs were prepared via solvent-thermal treatment of edible soybean oil, which generated glycerol-based polymer as a carbon source and fatty acid as a surface capping in the synthetic process. The as-synthesized CDs exhibited small particle size variation (<10%) and narrow emission bands (full width at half maximum <20 nm). The violet-emitting CDs (V-CDs) can self-assemble into blue-green-, yellow-, and red-emitting CD aggregates by increasing their concentration. Compared to commercial available organic dyes and semiconductor quantum dots, the CD aggregates provided 10-to-7000-fold improvement in brightness. Additionally, their emission wavelength was tunable across the entire visible spectrum by tuning excitation wavelength. Because of their high brightness, fluorescent imaging of single CD aggregate was simply achieved using filter-free dark-field fluorescent microscopy (DFM). We also demonstrate the use of filter-free DFM to dynamically image cellular uptake of V-CDs in the MCF-7 cells and Huh-7 liver cancer cells. Without the conjugation of fluorophore to the CDs, the particle aggregation-induced red-shift emission enables the development of CD-based ratiometric sensor for Fe(III) and pyrophosphate based on Fe(III) induced aggregation of V-CDs.
Bioanalytical - Fluorescence/Luminescence

Study on Effect of Structural Variation on Electronic Transitions, Photoluminescent, Non-Linear Optical Properties and Antimicrobial Activity of Pyrrole Hydrazones

For structure-properties variation study, pyrrole hydrazones (3A, 3B, 3C) have been synthesized by reaction of pyrrole precursor, and studied by FT–IR, 1H, 13C NMR, UV–Visible, emission spectra, DART Mass, Microanalysis and quantum chemical calculations. The calculated first hyperpolarizabilities ($\beta = 17.71, 48.83, 63.89 \times 10^{-30}$ esu) variation with structure found product 3C having more suitability for non-linear optical (NLO) applications. The gradual increase in $\beta$ values of products 3B, 3C compare to 3A is due to incorporation of electron withdrawing groups. The solvent-induced effects on the non-linear optical properties (NLO) were studied and found that as the solvent polarity increases, the $\beta$ value increases monotonically. The emission spectra show that the studied compounds are good photoluminescent material having intense emission at $\lambda_{em}$ 521 nm (3A, 3B) and 617 nm (3C) in visible (green and orange) region with Stoke’s shift 195, 160, 282 nm, respectively. The calculated thermodynamic and electronic descriptors analyses show products (3A, 3B, 3C) formation as exothermic and spontaneous at room temperature and have tendency to undergo hetrocyclization. The detailed vibrational analysis study carried out with the aid of potential energy distribution (PED) confirms variation in red shifts in N-H and C=O stretching bond as result of dimer formation and structural changes as result of incorporation of nitro and carbonyl groups. The preliminary bioassay shows that they (3A, 3B, 3C) have good antimicrobial activity. The Molar refractivity (MR) value of 3B (122.16 esu) is more than 3A and 3C and correlates well with experimental antimicrobial activity.

Keywords: Biosensors, Characterization, Spectrophotometry, Spectroscopy

Application Code: Bioanalytical

Methodology Code: Fluorescence/Luminescence
The process of programmed, cellular death known as apoptosis plays a central role in normal tissue homeostasis, and is implicated in the progression of numerous pathologies including cancer, neurodegenerative diseases, autoimmune diseases, and cardiovascular disease. Because of its involvement across this broad range of conditions, the development of techniques and platforms capable of selectively identifying apoptotic cells is of critical importance. Many approaches for the identification of apoptosis rely on characteristic morphological changes such as exposure of the membrane phospholipid phosphatidylserine (PS) on the cell surface. The protein Annexin V has been shown to selectively bind exposed PS in a specific manner with high affinity, and has been utilized previously for the detection of apoptotic cells by means of fluorescent or isotopic radio-labeling. We have begun the development a library of bioluminescent protein biosensors through the incorporation of Annexin V into a number of Annexin Fusion Proteins (AFPs) containing a bioluminescent reporter. In general, bioluminescence provides lower background and higher signal-to-noise ratios when compared to fluorescence, and does not carry the restrictive regulations on handling and disposal faced by isotopic labels. Moreover, these bioluminescent biosensors require no post-expression modification, and are capable of apoptosis detection both in vitro as well as in vivo. Incorporation of various photoproteins or bioluminescent enzymes with novel characteristics allows for specific tailoring of the resulting AFP. Among these reporters are RLuc8, a serum-stable variant of [i]Renilla[/i] luciferase; [i]Gaussia[/i] luciferase (GLuc), a very small (~20 kDa) protein with intensely bright emission; Aequorin, a photoprotein for which we have already developed tailored library of emission wavelengths and half-lives; and a truncated variant of [i]Vargula[/i] luciferase (tVLuc), which exhibits glow-type bioluminescence.

Keywords: Bioanalytical, Biomedical, Biosensors, Biotechnology
Application Code: Biomedical
Methodology Code: Fluorescence/Luminescence
To understand molecular mechanisms of protein reactions, it is important to study dynamics of intermolecular interactions. Here, we show time-resolved detection of a protein-DNA interaction by a diffusion based biosensor based on the transient grating method. By using this method, we can trace the intermolecular interaction spectroscopically in time-domain. We used a blue light sensor protein EL222 consisting of a light–oxygen–voltage (LOV) domain at the N-terminus and a helix-turn-helix DNA-binding domain at the C-terminus. EL222 acts as a light dependent transcriptional factor. To reveal this light-regulated molecular assembly mechanism, we studied the photoreaction of EL222 with a target DNA. Upon photoexcitation, EL222 showed adduct formation between the chromophore and the nearby Cys residue within few microseconds. After this reaction, conformation change and dimerization were observed as a reduction of the diffusion coefficient. Next, we added the target DNA in the EL222 solution. We successfully detected the DNA binding reaction to EL222 in millisecond time range as the decrease in the diffusion coefficient. A detailed analysis of the signal revealed that the DNA binding precedes the protein dimerization step. It was surprising to find that the reaction rate constant of the DNA binding was independent on the sequence of DNA fragments, although these fragments have different affinity to EL222. The result indicates that the protein-DNA binding affinity is governed by only the frequency of dissociation, not by the binding process.

**Keywords:** Biosensors, Laser, Protein

**Application Code:** Bioanalytical

**Methodology Code:** Sensors
Surface-enhanced Raman scattering (SERS) is an emerging technique for detection and identification of biological molecules and structures. SERS is considered as a potent method used for characterization, discrimination and identification of microorganisms by providing unique spectral fingerprints from microorganisms. Rapid, sensitive and accurate detection and characterization of bacteria is significant in many areas such as medical, environmental and industrial microbiology. In this study, SERS is used for the characterization of Methicillin-resistant Staphylococcus aureus (MRSA) and Legionella pneumophila isolates. Cultured bacteria are washed three times with water to remove the medium and metabolites of bacteria. The washed bacteria are mixed with concentrated silver nanoparticles (AgNPs) and the mixtures are dropped on a CaF2 slide. Then, SERS spectra are obtained from the mixture for the characterization of bacteria. The results demonstrated that, the obtained spectra can be used for the characterization of MRSA and L. pneumophila isolates.

Keywords: Bioanalytical, Biological Samples, Molecular Spectroscopy, Surface Enhanced Raman Spectroscopy

Application Code: Bioanalytical
Methodology Code: Vibrational Spectroscopy
Optical “virtual biopsy” is an attractive way to improve disease diagnosis and surgical guidance. Many optical microscopy techniques have been developed to provide diagnostic information without the need for tissue sectioning or staining. Among these techniques, label-free chemical imaging is the most desirable. Recently, it has been shown that narrowband, picosecond stimulated Raman scattering (SRS) can achieve comparable morphological contrast to hematoxylin and eosin staining (H&E staining), the ‘gold standard’ of pathology. However, to translate the technique from the bench to the bedside, optimal laser sources and parameters have yet to be identified. Here we describe an improvement to the narrowband SRS microscopy techniques for label-free tissue imaging. Through spectral slicing of broadband, femtosecond pulses, we are able to maintain the same protein/lipid contrast as narrowband SRS while achieving a higher signal-to-noise ratio (SNR). Our method draws upon the benefits of femtosecond pulses (i.e. higher peak power) while preserving those of picosecond pulses (i.e. adequate spectral resolution). We demonstrate this achievement through protein/lipid signal and contrast quantification of mouse brain tissue as a function of bandwidth, and comparison with numerical simulations. Further method validation is provided through imaging of additional mouse tissues: liver, kidney, and skin. The results of this work also informed our recent study into “virtual biopsy” of freshly excised human brain tumor tissue. We then color mapped the images to pseudo-H&E coloring to demonstrate the diagnostic capabilities of SRS imaging compared to traditional histopathology of the same tumor regions.
Reverse genetic strategies, such as virus-induced gene silencing, are powerful techniques to study gene function. Currently, there are few tools to study the spatial dependence of gene silencing at the cellular level. We report the use of multimodal Raman and mass spectrometry imaging to study the cellular-level biochemical changes that occur from silencing the [i]phytoene desaturase (pds)[/i] gene using a [i]Foxtail mosaic virus[/i] (FoMV) vector in maize leaves. The multimodal approach allows the localized carotenoid distribution to be measured and reveals differences lost in the spatial average when analyzing a carotenoid extraction of the whole leaf. The nature of the Raman and mass spectrometry signals are complementary: silencing [i]pds[/i] reduces the downstream carotenoid Raman signal and increases the phytoene mass spectrometry signal. Both techniques show that the biochemical changes from FoMV-[i]pds[/i] silencing occur with a mosaic spatial pattern at the cellular level, and the Raman images show carotenoid expression was reduced at discrete locations but not completely eliminated. The data indicate the multimodal imaging approach has great utility to study the consequence of gene-silencing at the cellular spatial level of expression.

Abstract Text
Reverse genetic strategies, such as virus-induced gene silencing, are powerful techniques to study gene function. Currently, there are few tools to study the spatial dependence of gene silencing at the cellular level. We report the use of multimodal Raman and mass spectrometry imaging to study the cellular-level biochemical changes that occur from silencing the [i]phytoene desaturase (pds)[/i] gene using a [i]Foxtail mosaic virus[/i] (FoMV) vector in maize leaves. The multimodal approach allows the localized carotenoid distribution to be measured and reveals differences lost in the spatial average when analyzing a carotenoid extraction of the whole leaf. The nature of the Raman and mass spectrometry signals are complementary: silencing [i]pds[/i] reduces the downstream carotenoid Raman signal and increases the phytoene mass spectrometry signal. Both techniques show that the biochemical changes from FoMV-[i]pds[/i] silencing occur with a mosaic spatial pattern at the cellular level, and the Raman images show carotenoid expression was reduced at discrete locations but not completely eliminated. The data indicate the multimodal imaging approach has great utility to study the consequence of gene-silencing at the cellular spatial level of expression.

Keywords: Imaging, Raman Spectroscopy
Application Code: Bioanalytical
Methodology Code: Vibrational Spectroscopy
Membrane receptors play important roles in regulating cellular activities. Targeting membrane receptors in cancer cells and understanding their interactions with specific ligands are key for cancer prognosis and therapeutics. However, there is a need to develop new technologies to provide molecular insight into ligand-receptor binding chemistry in cell membrane.

We have developed a tip-enhanced Raman spectroscopy (TERS) approach to study the ligand-receptor interactions. Using small molecule ligand-conjugated gold nanoparticles, we can target individual receptors both at substrate surfaces and cell membranes. By using a gold nanoparticle-attached TERS tip to scan the surface, we are able to obtain specific enhanced Raman signal associated with the ligand-receptor binding with a spatial resolution of tens of nanometers. We have shown that receptors with similar structures can be differentiated in intact cell membrane, according to the different Raman signal obtained with the TERS method. This methodology suggests a route to investigate the chemical interactions associated with ligand binding to membrane receptors in cells. In addition, we have demonstrated the capability of TERS not only to study the binding chemistry but also to provide information about binding specificity in intact cell membranes, which may improve early stage drug screening.

Keywords: Bioanalytical, Biospectroscopy, Chemometrics, Surface Enhanced Raman Spectroscopy

Application Code: Bioanalytical

Methodology Code: Vibrational Spectroscopy
Surface enhanced Raman spectroscopy (SERS) is a powerful technique that allows to acquire valuable label-free chemical information from complex biological samples for not only characterization but also diagnostic purposes. As a part of our ongoing effort to translate the technique into clinics for cancer diagnosis, we have comparatively studied several sampling methods using biopsied tissue samples. The sampling methods include by placing 5-µm cryosectioned tissue (typical thickness used in clinics) specimen on polydimethylsiloxane (PDMS) or CaF2, mixing homogenised tissue sample with colloidal Au+/AuNPs solution/suspensions, and using metabolites from the tissue sample. Each sampling method was studied for optimal experimental conditions to obtain the best S/N and spectrally rich SERS spectra. For the cryosectioned tissue samples placed on the surfaces, an aliquot of colloidal suspension was placed to cover tissue surfaces, and left to dry at either regular or suspended position. The SERS spectra collected from the several spots or an area scanned using rapid StreamHRTM scanning method were evaluated. Then, a wet chemical based in situ AgNPs synthesis method was developed in ultrasonic homogenization of tissue specimen by placing a volume of the mixture of homogenised tissue and Ag+/AgNPs/AuNPs solution/suspensions on a surface after a sonication process. In the final approach, the metabolites extracted from the tissue samples were used to collect SERS spectra. The collected spectra data was evaluated for spectral quality and reproducibility using principle component analysis (PCA)-based pattern recognition method, and accuracy as compared to the results of pathology. The comparative results indicate that all sampling methods provide high reproducibility, biochemical sensitivity, specificity, and classification accuracy (up to 100%).
The ability to visualize directly a large number of distinct molecular species inside cells is increasingly essential for understanding complex systems and processes. Even though existing methods have successfully been used to explore structure-function relationships in nervous systems, to profile RNA in situ, to reveal the heterogeneity of tumor microenvironments and to study dynamic macromolecular assembly, it remains challenging to image many species with high selectivity and sensitivity under biological conditions. For instance, fluorescence microscopy faces a ‘color barrier’, owing to the intrinsically broad (about 1,500 cm-1) and featureless nature of fluorescence spectra that limits the number of resolvable colors to two to five. Although surface-enhanced Raman scattering offers high sensitivity and multiplicity, it cannot be readily used to image specific molecular targets quantitatively inside live cells.

Here we engineered a novel class of alkyne-based dyes, for optical super-multiplexing. 20 distinct Raman frequencies are achieved through rational engineering of conjugation length, isotope doping and end-capping substitution. With further probe functionalization, we demonstrated unprecedented 10-color organelle imaging in single living cell with high specificity, sensitivity, and photo-stability. Moreover, optical data storage and identification are realized by combinatorial spectral barcoding, yielding the largest number of distinct barcodes to date. Therefore, these new dyes hold great promises in live-cell imaging and sorting, high-throughput diagnostics and screening, and information technology.

We acknowledges support from NIH Director’s New Innovator Award (1DP2EB016573) and R01 (EB020892), and the Camille and Henry Dreyfus Foundation.
Identification of foreign particles in tissue samples by pathologists has utilized a number of different techniques. Polarized light microscopy (PLM) is often a starting point to locate and describe a particle followed by additional measurements that may require isolation and mounting of particles for electron microscopy using an energy dispersive x-ray (EDS) detector to understand the elemental composition of the foreign material. Raman spectroscopy is well positioned to analyze mineral and inorganic oxide particles in tissue sections because it allows probing of small domain sizes, generally on the order of 1 micron, which means that individual particles that are incorporated into tissue can be analyzed discretely, with minimal interference from the surrounding tissue. The particle can also be analyzed without additional sample preparation, maintaining the relationship of the particle to the types of cells surrounding it which can provide information on tissue response. Examples of the identification of talc particles in lung and ovarian tumor sections, and metal wear particles from joint implants in liver tissue samples will be presented with a discussion of measurement conditions that lead to the best Raman spectral data for identification of the particles present.
In this study, gas chromatography and comprehensive two-dimensional gas chromatography (GCxGC) with TOFMS were employed to provide a mechanism of fingerprinting various cannabis strains based on differences in their terpene profiles. Cannabis is a complex mixture of compounds including cannabinoids, terpenes, terpenoids, non-cannabinoid phenols, nitrogenous compounds, flavonoids, and contaminants such as residual solvents and pesticides. The composition of cannabis is very important in determining its potency and medicinal effectiveness. An “entourage effect” is a synergistic relationship that exists between terpenes and cannabinoids in the plant. Analyses were performed using comprehensive chromatography and high performance time of flight mass spectrometry, as well as, effective data processing for characterization of samples. More specifically, GC and GCxGC-TOFMS profiling (fingerprinting) of plants was an effective way to determine the relative concentration of active components terpenes to provide unique component maps that could easily be compared via quick inspections. Instrumental analyses were relatively fast, reproducible and provided excellent chromatographic resolution. Deconvoluted mass spectral data were searched against well-established nominal mass libraries (e.g., NIST 17, Wiley 11) to facilitate confident identification of individual components in cannabis. These results were corroborated using additional software features such as advanced spectral filtering. Where possible, standards were analyzed for unequivocal confirmation of synergistic cannabis substituents.
Despite such accumulating evidence for potential negative health outcomes, no studies have been undertaken to evaluate the consequences to oral health due to eCigarette aerosol (EC-A) exposure. As EC technology, flavorings, illicit drug applications and its overall use are rapidly evolving in vaping (i.e., 'Cloud Chasers), evaluations are needed to inform the public and help provide evidence-based decisions for policy of EC’s. Specifically, tank and drip vaping, two popular vaping techniques. EC users can modify the devices to produce large vapor clouds, and thus potentially more toxicity. Recent studies demonstrate that EC-A’s may reduce glutathione levels in normal human oral keratinocytes and thus lead to cytotoxicity. The cytotoxicity is dose-dependent, but there is still no data on amounts of toxins absorbed by the cells. Most of the researchers’ expose cells to solution with captured EC-A; therefore, it is difficult to predict if the dose of the EC-A was chosen properly to demonstrate possible changes in the cells. The goal of our study is to develop a biorelevant in vitro mouth model, which will allow direct exposure and absorption onto human oral keratinocytes from EC-A. The model validation is ongoing for repeatability and uniformity of exposure using nicotine as a reference substance. In the next step of the study, the model is comparing the exposure for EC-A generated using different puffing topographies, which mimic three different methods of EC use (cartridge use, tank use, and drip vaping). We measured nicotine and carbonyl amounts in the cells simulating different EC use to compare the doses of toxins absorbed by the cells. Nicotine and carbonyls were extracted from the cell line using liquid/liquid extraction. The samples were analyzed using LC-MS/MS. Our results confirmed, that puff topography variations ultimately yield different cytotoxicity.
Cannabis is one of the most frequently encountered drugs among forensic toxicology laboratories. Forensic laboratories need to provide toxicological analyses, confirmation, and quantification of THC (tetrahydrocannabinol) and its primary metabolites THC-OH and THC-COOH in complex biological samples, including plasma and whole blood, for clients and enforcement agencies. Efficient extraction, cleanup, and quick analysis of samples submitted are extremely beneficial to the laboratory. The efficient extraction of THC and two major metabolites, THC-OH and THC-COOH, from plasma or whole blood is achieved using in-situ protein precipitation followed by a novel phospholipid removing filtration cartridge, Captiva EMR-Lipid, coupled with fast and quantitative analysis using LC/MSMS.

Sample preparation is an important consideration for forensic toxicology laboratories. THC and its metabolites can be prone to non-specific binding during sample preparation so an analytical method that eliminates multiple sample preparation steps, including off-line protein precipitation, centrifugation, and dilution but allows for streamlined in-situ protein precipitation with phospholipid removal is very desirable. This presentation will discuss a newly developed sample preparation product, Captiva EMR-Lipid, which is designed to remove more matrix interferences particularly phospholipids in a simple pass-through format, resulting in a cleaner extract, reducing problems with ion suppression, short column lifetime and MS source contamination in forensic studies.

Extraction details and the analysis of THC and its major metabolites, THC-OH and THC-COOH from biological samples using the Captiva EMR-Lipid cartridge, followed by LC/MSMS analysis will be presented. Analysis is rapid and highly reproducible, baseline separation with all analytes eluting in 3 minutes. Recoveries and RSDs were excellent and matrix effects (ME) were minimal for all compounds.

Keywords: Biological Samples, Forensic Chemistry, Liquid Chromatography/Mass Spectroscopy, Sample Preparation
Application Code: Clinical/Toxicology
Methodology Code: Sampling and Sample Preparation
Clinical and Toxicological Analyses

Analysis and Characterization of In Vitro Glutathione Adducts Formed with Drugs of Abuse

The human blood proteins hemoglobin (Hb) and serum albumin (SA) have free thiol groups that can be covalently modified by reactive chemical species. Such reaction products (“adducts”) are stable entities that have been utilized to examine exposure to xenobiotics. However, possible application of protein adducts to facilitate forensic toxicological detection of drugs of abuse has not been explored. This work examined the capability of various abused drugs to form adducts with the unbound cysteine thiol moiety of glutathione (GSH), and examined plausible structures for the adducts created. The drugs examined in this study cover a wide range of licit and abused drugs. Acetaminophen and clozapine, both of which have been previously reported to covalently adduct proteins in vitro and in vivo, were included as positive controls.

Instrumental analysis of GSH assay products was performed using negative mode ESI on both an Agilent 6460 LC-QqQ-MS and an Agilent 6530 LC-QTOF-MS. An Agilent Zorbax Rapid Resolution HD Eclipse Plus C18 column was used for separation. The total run time was 14 min with a 2 min post-run for column re-equilibration. Initial MS data analysis utilized Agilent’s MassHunter Qualitative Analysis software.

A total of 22 GSH adducts were observed for 11 of the 16 drugs examined in this study. These included a number of adducts not previously reported, including those for diazepam, oxycodone, and THC. Determination of plausible structures of the adducts involved in silico assessment of modifications of precursor drugs and commonly formed metabolites in combination with accurate mass data on molecular ions and major fragments.

Results demonstrated the capability of these drugs to covalently bind to thiol residues in vitro. Further determination of adducted species’ structures will aid in the ultimate creation of a validated method to analyze protein adducts formed by drugs of abuse as longer-term markers of exposure.

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Keywords: Drugs, Forensics, Liquid Chromatography/Mass Spectroscopy, Toxicology

Application Code: Clinical/Toxicology

Methodology Code: Liquid Chromatography/Mass Spectrometry
Amphetamine is an effective central nervous system stimulant. The control of this drug plays an important role in many fields, mainly clinical drug monitoring, doping, forensic applications and trafficking, attracting great importance to its detection. [1,2] Electrochemical methods provide a suitable sensitivity, easy portability and low cost for the detection of electroactive species. However, amphetamine is a non-electroactive compound [3] making its direct electrochemical detection impossible. In this work, we use a derivatization protocol between amphetamine and 1,2-Naphthoquinone-4-sulfonate (NQS) [4] to indirect electrochemical monitor the presence of this substance in urine samples using a portable paper-based analytical devices. In order to do that we fabricated a microfluidic paper-based analytical device combining filter paper and CO[2] laser scribed electrodes on paperboard. On the top of this device, the filter paper was molded with a sample spot (~200 µL) connected by two channels leading the solution into the detection zones by capillarity, and pretreatment/reaction zones in the midway delimited by wax pattern. In the detection zones there are 3 electrodes scribed by CO[2] laser under paperboard substrate. In one of the pretreatment zones, NQS was immobilized in bicarbonate buffer pH=9.0 to react with amphetamine and produce a new compound that can be monitored at -0.5 V after 5 minutes of reaction. In another zone, the urinary creatinine level was quantified as internal standard to detect adulterations/dilutions of the sample [5]. The proposed device allow an accurate, rapid and low cost detection of amphetamine abuse in urine samples at concentration lower than 30 µg mL[1].

Financial support: FAPESP, CAPES and CNPq.

References:
1. J. Heal. Sci. 2011, 57(6), 472.

Keywords: Derivatization, Electrochemistry, Forensic Chemistry, Lab-on-a-Chip/Microfluidics
Application Code: Clinical/Toxicology
Methodology Code: Electrochemistry
Clinical and Toxicological Analyses

Rapid and Easy Automated Sample Preparation with micro Solid Phase Extraction of Drugs from Blood and Biological Fluids

Abstract Text

Introduction & Aims:
Sample preparation, from extraction, concentration to dilution and isolation is vital for reliable and accurate analyses. Sample preparation is often the most time consuming physical job a chemist performs. Automation of sample preparation minimizes errors frees analysts from liquid handling tasks. Micro Solid-Phase Extraction (µSPE) is a new effective sample preparation technique using small bed volume and small (<3µm) particle size enabling smaller sample volume analyses than other forms of SPE along with advantages in speed, efficacy and solvent use. Blood is well known to be a complex and troublesome matrix that requires significant sample preparation, the use of µSPE has the potential to simplify this streamlining lab procedures.

Methods:
Using a mixture of atenolol, caffeine, acebutolol, propanolol, DXM, ketoconazole, verapamil and tolbutamide, an automated sample handling workflow was developed. This workflow incorporated processing of serum and spiked whole blood samples. The biological fluids were loaded onto a µSPEed cartridge, C18RPS-3µm/120Å (EPREP), washed with ultrapure water and then eluted using 20µl of meOH. All samples were prepared using the EPREP Sample Preparation Workstation prior to transfer to a Thermo Scientific Vanquish UHPLC system using a C18+ 100mm x 2.1mm 1.5µm column. Detection was performed via UV @ 210 and 254 nm and tandem mass spectrometry.

Result & Discussion:
The development of automated sample clean-up step for the analysis of a wide range of drug components streamlined the analysis workflow. Automated µSPE was successfully applied for the determination of drugs in serum samples, resulting in a rapid, effective and reproducible analysis with recoveries of (90.60% ± 6.20) for all analytes. The method has potential to replace and remove the need for complex sample preparation in the handling of whole blood using a simple and automated 3 step procedure.

Keywords: Automation, Drugs, Liquid Chromatography/Mass Spectroscopy, Solid Phase Extraction
Application Code: Clinical/Toxicology
Methodology Code: Sampling and Sample Preparation
Clinical and Toxicological Analyses

Microenvironment Attuned EGCG Modified NOCC Collagen Type I Scaffolds: A Case Study of Direct Participation of Osteoblast Seeded Scaffold in Healing Critical Sized Bone Defects

Neeraj K. Verma
CSIR-Indian Institute of Toxicology Research

Co-Authors: Aditya K. Kar, Amrita Singh, Mahaveer P. Purohit, Nitesh Dhiman, Satyakam Patnaik

Abstract Text

Low bone mass, osteoporosis (age-related and post-menopausal), and osteogenesis imperfects etc. causes bone fragility and increased the risk of fracture. To overcome these challenges, we intend to use adenosine, as an osteo-inductive agent for bone marrow-derived mesenchymal stem cells (MSCs) to differentiate them into functional osteoblast lineage. Subsequently, a suitable scaffold with tunable physico-chemical attributes were developed using a cocktail of N, O-carboxymethyl chitosan (NOCC), Epigallocatechin Gallate (EGCG) and Collagen Type I to mimic the chemical and mechanical environment of natural bone along with providing osteo-inductive cues. The ratios of scaffolding materials in the cocktail were optimized based on their physico-chemical properties priming them for their osteo-inductive and osteoconductive abilities. The synthesized scaffolds were thoroughly characterized by standard techniques. To assure successful synthesis, surface morphology and chemical composition of scaffolds was assessed by scanning electron microscopy (SEM), FT-IR and NMR. These optimized scaffolds reveals a highly porous network structure with rapid swelling, enhance water retention and adequate tensile strength. These scaffolds showed antimicrobial activity against pathogenic microbes. For in-vitro studies, MSCs differentiated osteoblasts seeded scaffolds were characterized by using cell viability, alkaline phosphatase activity, Immunocytochemistry, western blotting and matrix mineralization assays etc. Our preliminary findings further substantiate the efficacy of the optimized scaffolds which will be assessed in-vivo by creating calvarial bone defect in a murine model by using different molecular and histo-pathological assays. With the above background, we hope that our approach may culminate towards a new and better scaffolding material for fractured bone healing in the near future.

Keywords: Biomedical, NMR, Toxicology, UV-VIS Absorbance/Luminescence
Application Code: Biomedical
Methodology Code: Process Analytical Techniques
Fluorescence/Luminescence

High Resolution Fluorescence Spectroscopy for the Analysis of High Molecular Weight Polycyclic Aromatic Hydrocarbons in Liquid Chromatographic Fractions and Sample Extractions of Environmental Samples

This presentation focuses on the detection and characterization of high-molecular weight polycyclic aromatic hydrocarbons (HMW-PAHs), i.e. PAHs with molecular weight (MW) equal or higher than 302 g mol⁻¹. Some PAH isomers of MW 302 have shown higher mutagenic activity than the sixteen PAHs included in the priority pollutants list of the U.S. Environmental Protection Agency (EPA-PAHs). Individual isomers of HMW-PAHs are not routinely identified or quantified. Difficulties in the determination of HMW-PAHs arise from their low concentration levels in environmental samples compared to those of the EPA-PAHs. The number of isomers increases dramatically with each additional aromatic ring, which makes separation and identification difficult by gas chromatography-mass spectrometry and liquid chromatography. Herein, we demonstrate our ability to differentiate individual PAH isomers of MW 302 on the basis of their low-temperature (77K and 4.2K) fluorescence spectra. Vibrational resolution with fingerprint spectral information is obtained by dissolving HMW-PAHs in n-octane. Quantitative analysis at the parts-per-billion concentration level (ng.mL⁻¹) is made possible with no need of sample pre-concentration. Strategies are presented for the analysis of sample extracts and chromatographic fractions of complex environmental samples.

Keywords: Fluorescence, PAH
Application Code: Environmental
Methodology Code: Fluorescence/Luminescence
Fluorescence/Luminescence

Virtual-Instrument-Controlled Apparatus for Rapid Collection of Fluorescence Excitation-Emission Matrices (EEMs)

The qualitative and quantitative measurement of polycyclic aromatic compounds (PACs) in environmental samples through their characteristic fluorescence requires that one distinguish sometimes very similar luminescence data. PAC isomers can have similar absorption and emission spectral features, so for increased specificity one measures a multi-dimensional map of a molecule's fluorescence in the form of a time-resolved excitation-emission matrix (TREEM). This creates a four-dimensional fingerprint for that PAC. Cooling samples to liquid nitrogen or liquid helium temperatures narrows their spectral features through the Shpol’skii effect, providing even more distinction. A TREEM-collection instrument dubbed the “TREEMWeaver” that combines all these techniques has been constructed from separate commercial products, which are centrally controlled through a LabView virtual instrument. A pulsed, wavelength-tunable laser provides a light source that allows the observation of the fluorescence time dependence. An imaging spectrograph and intensified CCD resolve the emission spectra and allow a modest improvement in rates of data collection over the scanning monochromators used in most commercial spectrofluorimeters. We discuss the factors that must be monitored and controlled to ensure accuracy and reproducibility of results with such a device. The authors acknowledge financial support from The Gulf of Mexico Research Initiative (Grant 231617-00). The views expressed are those of the authors and do not necessarily reflect the view of this organization.

Keywords: Environmental Analysis, Fluorescence, Instrumentation, Spectroscopy
Application Code: Environmental
Methodology Code: Fluorescence/Luminescence
## 4-Color Logic Gate Based on DNA-assembled Photonic Wire - FLASH PRESENTATION

We know multicolor fluorescence analysis suffers from severe spectral crosstalk in the visible spectrum. A linear photonic wire assembled by a DNA scaffold provides Förster resonance energy transfer (FRET) for fluorescence analysis will be discussed. The photonic wire takes advantage of spectral overlap to sequentially transfer the energy through the photonic wire. The multicolor fluorescence system will rely on FRET indices that are relative values determined by the ratio of the acceptor to donor emission intensity. We will address a synchronous scanning approach to mitigate the spectral crosstalk on excitation and emission to measure the fluorescence of 4 fluorophores, Cy3, Cy3.5, Cy5, and Cy5.5, arranged in the DNA assembly. Applying specific excitation and emission wavelengths for each dye minimizes the spectral crosstalk for determining the FRET indices between the dyes. The FRET indices depend heavily on the fluorophores' distance from each other and spectral overlap integral of the FRET dyes present in the assembly. A systematic investigation on the potential fluorophore combinations using both dye-labeled and unlabeled DNA scaffold shows the collection of FRET indices from the combinations. Putting the collection together, the signature of the FRET indices from each combination could be employed to identify the presence of fluorophore in the assembly. The 4-color logic gate has a potential for molecular computing, multicolor fluorescent probe, and biosensing. The flexible fluorescence measurement strategy by synchronous scanning will benefit the DNA assembled photonic wire to detect the presence of single dyes, FRET pair, and FRET cascade.

**Keywords:** Bioanalytical, Fluorescence, Nucleic Acids, Validation

**Application Code:** Bioanalytical

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

Langmuir films and Fourier Transform Infrared (FTIR) spectroscopy were used to elucidate the interaction between the pesticides picloram and glyphosate with ternary mixtures composed by phospholipid 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC), sphingomyelin (SM) and cholesterol (Chol). The \( A \) isotherms were recorded by spreading the DOPC/SM/Chol at a 1:1:1 molar ratio both in ultrapure water and picloram or glyphosate aqueous solution. The isotherm of pesticides-DOPC/SM/Chol combinations reveal an expansion of the mean molecular area, indicating that both pesticides interact with the monolayer and possible miscible with the ternary mixture. In the first case, the isotherm of the mixed film with glyphosate shifted to higher area which is the consequence of the formation of mixed monolayer at the air/water interface where the glyphosate molecule between the lipid molecules occupy an additional area. We suppose that this behavior attributed to the strong affinity of glyphosate to the ternary mixture due to its multiple ionizable groups. The DOPC/SM/Chol mixture affinity for glyphosate is higher than (approx. 5 times) that for picloram. In addition, FTIR spectroscopy technique confirmed that pesticides could induce perturbation around the acyl chains and head-group region of the mixture suggesting some level of interaction. These results support the understanding of pesticides-multicomponent lipid bilayer, as well as the complex model of cell membrane.

Keywords: Fluorescence, Infrared and Raman, Lipids, Pesticides

Application Code: Biomedical

Methodology Code: Fluorescence/Luminescence
Data integrity compliance has become one of the most important components of industry’s responsibility to ensure the safety, efficiency and quality of drugs to protect human health. However, regulatory agency has increasingly observed Current Good Manufacturing Practice (CGMP) violations related to data integrity during inspections in recent years. This is because not only the role of data integrity on the CGMP requirements is not well understood, but the scope of data integrity is more than expected before in the analytical laboratory.

Regulatory agency expects that data must be reliable and accurate on the CGMP criteria even for the stand-alone computerized laboratory instruments such as UV and FT-IR instruments to the same level of chromatographs. As the stand-alone computerized laboratory instruments have data management system individually, it forces to complicated operational workflow, and increases human error risk and system maintenance cost. A unified network platform for the various instruments that supports centralized user access control, user rights and system policies, integrated data management and audit trail is a solution for the regulated analytical laboratory. The Report Set function enables the visibility of the analytical laboratory that automatically combines a set of reports for each sequence, such as Data Reports, Acquisition & Processing method reports, Sequence logs and audit trail information and then digitally consolidates them into a single PDF file. This feature provides traceability of analysis operations. In this presentation, it is shown how data integrity compliance is effectively achieved on the unified network platform in the analytical laboratory.

Keywords: Lab Management, Laboratory Informatics
Application Code: Laboratory Management
Methodology Code: Laboratory Informatics
Implementing new laboratory informatics software or systems can be very challenging and can be very rewarding. Many data points, headcount, finances, process flows, procedures, and more should be evaluated before beginning a lab informatics software implementation. Often times, employees are assigned to an internal project team to review processes, evaluate what available resources can support a project, and then pick an informatics software that they think will resolve their perceived issues, purchase the software, and try to implement. Sometimes the implementation goes well, most of the time it runs into a lot of issues and ends up taking 2-4 times as long as it should.

Needs Assessments are a critical element to planning and delivering projects in the most efficient way possible. Needs assessments allow organizations to evaluate resources, processes, inefficiencies, variance in process execution, systems to be interfaced, and more. They allow the company to standardize processes, understand the true needs from the lab software, select the appropriate solution, and implement much faster while removing wasted effort. Needs Assessments should be performed by unbiased individuals in order to observe gaps that may exist that the group owners do not see as they have become adjusted to the current process and may not recognize the gap. Performing Needs Assessments identify workflows, pain points, quality risks, resource deficiencies, and future state process maps. Needs Assessments allow implementation teams to prepare better for known roadblocks needing mitigation, required resources to execute the implementation, define level of effort more accurately, and standardize previously variant processes. In performing Needs Assessments, the return on investment is realized sooner due to the most optimized implementation in the shortest amount of time to deliver the right-fit software solution.

Keywords: Lab Management, Laboratory Automation, Laboratory Informatics, LIMS
Application Code: Laboratory Management
Methodology Code: Laboratory Informatics
Laboratories are constantly striving to move more and more to a fully automated state. Instruments are capable to interface with Laboratory Information Management Systems (LIMS), with Electronic Laboratory Notebooks (ELN), and with acquisition software that also be integrated with LIMS systems. As customers continue to purchase new automated solutions, one of the most frequent questions asked is, “Where will I put all of the computing hardware considering my already overcrowded benches?”. Bench real estate is a constant challenge with purchase of new instrumentation, hardware, and growth in the quantity of testing analysts requiring sample preparation and analysis space.

Fortunately, LIMS providers are now growing in their technology to provide mobile result entry options. Some LIMS are allowing users to leverage mobile devices such as smart phones, tablets, and laptops to enter results directly into their LIMS database via web browser and the ability to connect to the internet. By facilitating collection of data away from workstations, LIMS mobile result entry solutions reduce the time for data to be returned to the LIMS, improve data quality, and eliminate the need for paper forms, providing a rapid return on investment.

Keywords: Lab Management, Laboratory Automation, Laboratory Informatics, LIMS
Application Code: Laboratory Management
Methodology Code: Laboratory Informatics
When it finally becomes time to upgrade your home brewed collection of paper and electronic files or existing Laboratory Information Management System (LIMS) where do you start and what do you look for? How do you avoid becoming a laboratory that invested in a LIMS and is still unable to use it?

Purchasing a LIMS for the first time can be an overwhelming experience for any laboratory. There are so many choices to choose from and products that are ideal for some market sectors but may not be a good fit for others. This presentation is focused on providing information to buyers (both for first time and experienced organizations) and helping them understand the process they are about to undertake. Information included in the discussion will help translate some of the LIMS “jargon” and allow the lab to make an informed decision.

Topics will include:
- A step-by-step review of the LIMS planning and implementation process that begins with defining the project scope and ends with final deployment and going live.
- Why a needs assessment is a laboratory’s secret weapon to a successful LIMS selection and implementation project.
- How to properly define your LIMS requirements for both today and tomorrow.
- LIMS and the Cloud: A growing deployment option that opens the door to a LIMS for many organizations.
- A collection of the most valuable best practices and lessons learned that will help attendees avoid obstacles to a successful LIMS deployment project.

Keywords: Lab Management, Laboratory Automation, Laboratory Informatics, LIMS
Application Code: Laboratory Management
Methodology Code: Laboratory Informatics
Abstract Text
The wide variety of analytical systems and types of electronic data present significant challenges for bioanalytical laboratories that are required to maintain data integrity and long term data readability. Proprietary instrument data formats are often unreadable after time, as the necessary software no longer runs on current operating systems. The Analytical Information Markup Language (AnIML) is the ASTM open data standard that defines XML tagging for any kind of analytical information. AnIML supports vendor independent and technology neutral analytical data exchange. It also captures audit trails and digital signatures for improved compliance. Conversion or export to AnIML allows generic viewers to access data after proprietary software is no longer supported or operational. This presentation will focus on technologies that enable data integrity, regulatory compliance, and long term data storage. It explains how combining instrument and study data in AnIML format can enable a comprehensive bioanalytical study data archive.

Keywords: Bioanalytical, Laboratory Informatics, LIMS, Scientific Data Management
Application Code: Bioanalytical
Methodology Code: Laboratory Informatics
G-quadruplex DNA Protein Capture from Matched Normal and Tumor Human Tissues

The role of three-dimensional genomic architecture in gene regulation has become a topic of intensive investigation in recent years. In particular, there has been extensive speculation about possible roles of in vivo G-quadruplex (G4) formation in nuclear processes. G4 structures are secondary structures arising from formation of two or more hydrogen-bonded guanine tetrads in certain G-rich sequences. Interest has been fueled by discovery of ever-increasing numbers of G4-forming genomic sequences with putative links to genomic function and of proteins that exhibit some function or activity related to G4 structures. We have been studying in vitro affinity protein capture by G4-forming sequences from the human genome. Proteins are identified by using mass spectrometric analysis followed by Western blotting to confirm the identifications. Here we describe protein capture from matched normal and tumor tissue samples by several G4-forming sequences from oncogene promoter regions, in order to determine differential protein expression related to cancer. The goal is to obtain new insight into cancer gene regulation that will lead to earlier diagnosis and identification of new therapeutic targets.

Abstract Text

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Keywords: Bioanalytical, Genomics, Nucleic Acids, Protein

Application Code: Bioanalytical

Methodology Code: Mass Spectrometry
Derivatization of Polysaccharides to Facilitate Multiply-Charged Ion Formation for Electrospray Ionization Mass Spectrometric Analysis

Analysis of polysaccharides by mass spectrometry continues to evolve slowly due to their low ionization efficiency. Compared to advances in protein analysis using MALDI- and ESI-TOF MS, improvements in ionization for MS analysis of polysaccharides have moved rather slowly. Some derivatizations have been utilized to improve MALDI-TOF analysis with moderate success. In contrast, electrospray ionization has been generally regarded as unsuitable due to multiple-charged ion formation, lower ionization efficiency of neutral polysaccharides, and the spectral complexity generally observed. Instead of seeing ESI as an unsuitable ionization technique, we took advantage of the multiply-charged ion aspect to analyze derivatized dextran. To improve ionization and encourage multiple charges on an otherwise neutral molecule, a one-pot derivatization of dextran was performed to attach free-amino containing groups following a procedure that has been previously used to generate water-soluble Taxol conjugates with dextran. After applying the derivatization, the isolated product was analyzed using an Agilent 6224 TOF LC/MS equipped with a dual-ESI ion source by direct infusion. Following the success of this derivatization with dextran-1 (~1 kDa), we have investigated its applicability to larger molecular weight dextran-3.5 and dextran-6. With these experiments, the expectation is to be able to analyze large polysaccharides in a similar manner that multiply-charged proteins are analyzed using mass spectrometry. Once the experiments are completed, we will determine if this derivatization is applicable to perform high molecular weight measurements of polysaccharides, which may allow for structural elucidation of large polysaccharides in the future.

Keywords: Bioanalytical, Electrospray, Mass Spectrometry, Method Development
Application Code: Bioanalytical
Methodology Code: Mass Spectrometry
Chitosan is a derivative of chitin, the second-most abundant polysaccharide found in nature. It is composed of randomly distributed glucosamine and N-acetyl glucosamine subunits. Chitosan is a cell wall component of many species of fungi. Given its ubiquity in prevalent pathogenic species of fungus, chitosan has the potential to serve as an indicator of fungal infections. Polymeric chitosan detection is difficult using mass spectrometry due to the limited ionizability of polysaccharides. Facets of method development will be discussed with the goal of detecting various lengths of chitosan using mass spectrometry. One of these is the characterization of chitosan oligomers using electrospray ionization time-of-flight mass spectrometry. Another is the degradation of polymeric chitosan to create a series of oligomers, thereby allowing mass spectrometric detection of fungal-derived chitosan. Detection of varying lengths of the underativized polysaccharide will be covered, with the effects of pre-treatment steps, mobile phase compositions, and injection methods taken into account. Successful mass spectrometric detection of chitosan as a diagnostic tool for fungal infections will accomplish two things by (1) giving a nonspecific screening method against fungal infection, and (2) providing an early detection method for fungal infections.
The processes of oxidation and neuroinflammation of the brain has been suggested as possible cause to the development of Alzheimer’s disease (AD), with changes in the brain occurring years or possibly decades prior to the onset of cognitive impairment. Blood and cerebrospinal fluid (CSF)-based markers have been shown to discriminate AD patients 15 and 25 years respectively prior to cognitive dysfunction. Free fatty acids are highly oxidisable and have shown to be important mediators of inflammation in the brain. The aim of this study therefore was to investigate the minimum sample amount required to detect free fatty acids as potential biomarkers of AD in blood plasma and CSF. Eight sample volumes were tested in the range 50 – 200 [micro]L for both plasma and CSF. This study investigated the use of pentofluorobenzyl bromide derivatization of free fatty acids isolated from blood plasma and cerebrospinal fluid, for analysis by GC-MS. The method was developed on the Agilent 7200 GC-QTOF-MS operated in negative chemical ionisation mode. Data were processed using AnalyzerPro v5.4. Twenty-five fatty acids were identified in plasma and 11 in CSF. This method has been shown to be a robust and sensitive measure of free fatty acids from mammalian biofluids. The methodology will likely permit the measurement of free fatty acids at femtogram amounts.

Keywords: Biological Samples, Gas Chromatography/Mass Spectrometry, Metabolomics, Metabonomics
Application Code: Biomedical
Methodology Code: Mass Spectrometry
Direct Sampling of Sub-µm Atmospheric Particulate Organic Matter in Sub-ng m-3 Mass Concentrations by Proton-transfer-reaction Mass Spectrometry

A quantitative characterization of the organic fraction of atmospheric particulate matter is still challenging. Herein we present the novel modular "Chemical analysis of Aerosol Online" (CHARON) particle inlet system coupled to a new generation proton-transfer-reaction time-of-flight mass spectrometer (PTR-TOF 6000 X2, Ionicon Analytik, Austria) that quantitatively detects organic analytes in real-time and sub-pptV levels by chemical ionization with hydronium reagent ions. CHARON consists of a gas-phase denuder for stripping off gas-phase analytes (efficiency > 99.999%), an aerodynamic lens for particle collimation combined with an inertial sampler for the particle-enriched flow and a thermodesorption unit for particle volatilization prior to chemical analysis. With typical particle enrichment factors of around 30 for particle diameters (DP) between 120 nm and 1000 nm (somewhat reduced enrichment for 60 nm < DP < 120 nm) we boost the already excellent limits of detection of the PTR-TOF 6000 X2 system to unprecedented levels. We demonstrate that particulate organic analytes of mass concentrations down to 100 pg m-3 can be detected on-line and in single-minute time-resolutions. In addition, PTR-MS allows for a quantitative detection of almost the full range of particulate organics of intermediate to low volatility. With the high mass resolution (> 6000) and excellent mass accuracies (< 10 ppm) chemical compositions can be assigned and included in further analyses.

In addition to a detailed characterization of the CHARON PTR-TOF 6000 X2 we will present first results on the chemical composition of sub-µm particulate organic matter in the urban atmosphere in Innsbruck (Austria).

Keywords: Aerosols/Particulates, Chemical Ionization MS, Environmental Analysis, Mass Spectrometry
Application Code: Environmental
Methodology Code: Mass Spectrometry
Since the inception of ESI, the method development and optimization has been difficult for the experts and newcomers. As empirical knowledge on both the analytes/solutes and the solvents/eluents/additives/matrices has grown, coming up with a “from scratch” analytical ESI method has become more accessible for the experts but has still remained largely a trial-and-error for the novices. The issue is exacerbated by the interdependencies of the method parameters and intrinsic variability between various ion sources (especially from different instrument models and even more so between different manufactures), in stark contrast to the electron ionization (EI). Thus, a newcomer to IC/LC-MS field often lacks the possibility of “borrowing” a published method (even for the same analyte and mobile phase) and using it as a reliable starting point on a different instrument. Heated electrospray (HESI) method optimization cannot be reduced to partial derivatives in the method parametric space and remains a tedious task even for the experts in the field, despite the fact that such optimization is routinely and frequently required in the lab.

We will present our attempt to significantly simplify the issue of de novo HESI method development in IC-MS and LC-MS for the novices by (a) providing a reliable, empirical data-based starting point (plateau range) for a given ion source; (b) developing algorithms that interlink the method parameters with each other and, more importantly, with the desirable analytical figures of merit and the physico-chemical characteristics of the analytes and the mobile phase, intuitively assessable by any chemist; (c) wrapping those algorithms into an easy-to-adopt and easy-to-use graphical user interface of the ISQ EC instrument method editor that allows novices not only to create a working HESI method from scratch but also educates them on the individual method parameters values and brings to the next level of expertise in IC/LC-MS.

Keywords: Electrospray, Ion Chromatography, Mass Spectrometry, Method Development
Application Code: Environmental
Methodology Code: Mass Spectrometry
Eugenol, the main composition of clove and clove oil, is a colorless or pale yellow liquid that can be used for antibacterial agent, hypotensive agent, and food additives. It is widely used in aquaculture for its anesthetic action. However, studies have shown that consumption of eugenol-containing fishery products may lead to skin allergy or liver damage. This potential hazard of eugenol has given rise to intensive investigation of its detection.

Analytical methods currently available for the detection of eugenol mainly include TLC, HPLC, GC-MS/MS, HPLC-MS/MS, etc. An analytical method was proposed in this paper for determination of eugenol in carp, catfish and shrimp matrices with Shimadzu GCMS-TQ8040 triple quadrupole gas chromatograph-tandem mass spectrometer.
Pancreatic islets play a key role in maintaining glucose homeostasis by dynamically secreting regulatory hormones, most prominently insulin and glucagon. Impairment in the release profile of these hormones is a hallmark of the diabetic state. While robust methods for monitoring insulin secretion exist, an approach to measure glucagon is still lacking. In this work, an approach is presented to measure glucagon with single islet sensitivity and with the ability to perform continuous measurements. A microfluidic noncompetitive immunoassay scheme using a fragmented antibody labeled with fluorescein isothiocyanate (Fab*) was developed for measuring glucagon. Noncompetitive immunoassays use an excess of Fab* to chelate all the glucagon in solution, reducing the dependence of the equilibrium binding constant. The limit of detection (LOD) for these assays is limited by the LOD of the optical system, typically sub-nanomolar. To improve the LOD further, a confocal single point detection system is preferred due to its sensitive detection capabilities and high signal-to-noise ratio. A focusing lens was used to focus the collimated emission light onto a pinhole before the PMT. To test the system, different size of pinholes, 400, 100, and 50 [micro]m, were used for the optimization of the z-axis resolution. Once the system if fully developed, the limit of detection of the noncompetitive immunoassay will be...
Fluid mixing in microfluidics is a fundamental fluid operation for many applications, such as chemical kinetic studies, gradient generation, diagnostic assays, and nanomaterial synthesis. It is not a trivial task to mix different fluid flows as viscous forces typically dominate over inertial forces in microfluidics. To overcome the inherent challenge, many methods have been reported based on either passive fluid flow manipulation or active external force field. Among the existing methods, sharp-edge enabled acoustic based mixing shows unique advantages in terms of short mixing time (<1 s) and minimum footprint and hardware demand, which makes it an ideal method for applications requiring both fast mixing kinetics and portability. However, a major roadblock for further adoption of this method for practical applications is the noise generated during the device operation as the working frequency is in the audible range (~4.7 kHz). Here, we developed a new generation of acoustic mixing device by exploiting the boundary driven streaming instead of the streaming generated from vibrating sharp-edges. As a result, we were able to operate the device at a different frequency while maintaining the same performance as the sharp-edge method. Through optimizing the geometry of microstructures, we successfully mixed two fluid flow within 8 seconds with a non-auditable working frequency (95 kHz). The new “silent” acoustic mixing method eliminates the noise problem of current method, and will, therefore, accelerate the adoption of this methods by the community.

Keywords: Lab-on-a-Chip/Microfluidics, Nanotechnology
Application Code: Bioanalytical
Methodology Code: Microfluidics/Lab-on-a-Chip
Development of a Microfluidic System for Monitoring Glucagon Secretion From Islets of Langerhans

Glucagon is a hormone released from pancreatic islets of Langerhans that stimulates glucose release by the liver and plays a critical role in maintaining glucose homeostasis. Glucagon measurements from single islets are of interest because its release is thought to be pulsatile and out of phase with insulin, although this has not been determined due to a lack of analytical assays with appropriate sensitivity and time resolution.

To achieve quantitative monitoring of glucagon secretion from single islets, a noncompetitive assay for glucagon has been developed using a fluorescently-labeled aptamer (Apt*) as the binding agent which is maintained at 250 nM. The free and bound Apt* are separated by capillary electrophoresis and detected by laser-induced fluorescence, producing detection limits of 6 pM. A microfluidic device was developed to automate this assay and make it suitable for online monitoring. To achieve this goal, the Apt* is delivered to the device using a pressure-driven system and mixed with islet perfusate as it travels to a separation channel. The reagents are then injected into the separation channel and detected 3-cm downstream from the injection cross. The use of the pressure system to drive solution flow down the mixing channel enables any buffer to be used and produces unbiased injections. The channel dimensions were optimized to obtain an injection volume that was reproducible with RSDs < 5%. This system will be suitable for monitoring glucagon release from single islets to enable determination of secretion dynamics.

Abstract Text

To achieve quantitative monitoring of glucagon secretion from single islets, a noncompetitive assay for glucagon has been developed using a fluorescently-labeled aptamer (Apt*) as the binding agent which is maintained at 250 nM. The free and bound Apt* are separated by capillary electrophoresis and detected by laser-induced fluorescence, producing detection limits of 6 pM. A microfluidic device was developed to automate this assay and make it suitable for online monitoring. To achieve this goal, the Apt* is delivered to the device using a pressure-driven system and mixed with islet perfusate as it travels to a separation channel. The reagents are then injected into the separation channel and detected 3-cm downstream from the injection cross. The use of the pressure system to drive solution flow down the mixing channel enables any buffer to be used and produces unbiased injections. The channel dimensions were optimized to obtain an injection volume that was reproducible with RSDs < 5%. This system will be suitable for monitoring glucagon release from single islets to enable determination of secretion dynamics.

Keywords: Electrophoresis, Fluorescence, Lab-on-a-Chip/Microfluidics, Peptides
Application Code: Bioanalytical
Methodology Code: Microfluidics/Lab-on-a-Chip
Microchip-based devices have made it possible to perform rapid separations with high temporal resolution. These chips can also be integrated with cell culture to analyze cellular components. Bilayer PDMS devices can be used to separate electrophoresis buffers from high ionic physiological buffers along with an injection scheme using pneumatic valves and pumps. Our lab has shown that these devices can be sealed onto a polystyrene substrate that can be integrated with an electrode and a Pd decoupler for electrochemical detection. Due to poor electrophoretic performance of PDMS, separation resolution is often limited with these devices. The effectiveness of microchip devices can also be limited if cells are immobilized on-chip using a 2D substrate. In this talk, we will show that fused silica capillaries can be embedded into PS along with electrodes to facilitate higher efficiency separations on-chip along with a technique for integrating modular 3D cell culture to mimic an in vivo environment. Fluorescence detection was used to show loop-based hybrid devices can achieve higher efficiency separations when compared to traditional PDMS serpentine device of the same length. The embedded devices have also been combined with electrochemical detection for analysis of a complex separation catecholamines along with K\textsuperscript{+} stimulated PC12 cell release of DA and NE. PS fibers have been used with PS sheets through the use of “wind-spinning” and laser cutting to create pallets for cellular immobilization. PC12 cells have been immobilized on these fibers after coating them with collagen solution. A 2-reservoir design has been used resulting in a decrease in the disturbance of the cells when introducing stimulants. Using a fused silica capillary on-chip along with pallets of fibers opens up new avenues of research for high efficiency electrophoretic separations on microchip devices, while also providing a method for high throughput analysis of in vivo mimics.

Keywords: Bioanalytical, Electrochemistry, Electrophoresis, Lab-on-a-Chip/Microfluidics
Application Code: Bioanalytical
Methodology Code: Microfluidics/Lab-on-a-Chip
Membrane proteins are an important class of proteins with essential roles in a myriad of cellular processes including intercellular communication, energy production, biomolecule transport, and signaling cascades. When outside of their native lipid bilayer membrane proteins tend to misfold or precipitate from aqueous solutions, necessitating the development of model membrane systems. Using lipid self-assembly and a scaffold protein, Nanodiscs offer a tunable, stable bilayer structure that form spontaneously upon the removal of a solubilizing detergent. Membrane proteins can be incorporated into Nanodiscs and have been shown to maintain activity across many membrane protein classes. An analytical need to reduce Nanodisc assembly time and reagent consumption and to optimize membrane protein incorporation into Nanodiscs led to the development of microfluidic devices for rapid and efficient Nanodisc self-assembly and membrane protein incorporation.

Beyond incorporation of isolated membrane proteins into Nanodiscs, on-chip Nanodisc self-assembly beginning with cell lysate as a starting material can produce libraries of Nanodiscs containing membrane proteins that accurately represent the membrane protein composition of the starting material (e.g. cells, tissue). To study just one of these membrane proteins requires a purification method to remove the Nanodiscs containing other proteins, membrane molecules and empty Nanodiscs. We have implemented Nanodisc library generation and target-specific Nanodisc purification onto a microfluidic platform. The platform consists of a library generation and purification modules that output Nanodisc libraries containing only protein targets of interest from the full membrane protein library. This library preparation and purification will allow the study of a specific and active membrane protein of interest isolated from a cell in a sample that is too small for normal protein purification processes, such as fine needle aspirate biopsies.
Developing a Droplet Microfluidic Toolbox using Mass Manufacturable Thermoplastic Material Systems

Droplet microfluidic devices have emerged as promising tools for a number of bioanalytical applications—particularly those that stress small volume analyses and minimal sample loss. A large majority of droplet microfluidic devices have been based on poly(dimethylsiloxane) (PDMS) made via rapid prototyping in academic laboratories. However, there has been little progress in adapting these droplet manipulation technologies to more mass producible material systems, such as thermoplastics. This presentation will describe recent progress in developing new microfabrication procedures to manufacture droplet microfluidic devices in poly(methyl methacrylate) (PMMA). This process involves hot embossing with silicon templates fabricated using photolithography, wet etching, and deep reactive ion etching techniques. The resulting device quality was characterized with profilometry and electron microscopy techniques. Furthermore, we employed a PMMA oxidation procedure to hydrophobically modify channel walls to reduce wall wetting in order to generate droplets of controllable size, frequency, and stability. These new PMMA devices are being tested for important microfluidic operations including droplet generation using T-junction and flow-focusing configurations, the K-channel for controlled volume addition and removal, and delay-channels for on-chip droplet incubation. As these component designs are optimized we will integrate them into a suite of droplet microfluidic tools that our laboratory is developing for applications in low sample input epigenetic analysis.

Keywords: Bioanalytical, Lab-on-a-Chip/Microfluidics, Method Development, Microscopy
Application Code: Bioanalytical
Methodology Code: Microfluidics/Lab-on-a-Chip
Microfluidics/Lab on a Chip

Electrophoretic Methods for Monitoring Reactive Nitrogen Species

In the push to develop treatments for neurodegenerative diseases, a central goal is to probe the chemistry underlying these conditions. It has been proposed that nitrosative stress resulting from the accumulation of reactive nitrogen species (RNS) in the brain may contribute to the formation of beta-amyloid plaques in Alzheimer’s disease. Tyrosine residues are often nitrated in the presence of the RNS peroxynitrite, while glutathione undergoes an oxidation reaction to form glutathione disulfide. These chemical changes can have damaging effects on cellular function. The ultimate goal of this project is to develop methods to probe these reactions in cells in order to better understand neurodegeneration. In order to do this, peroxynitrite was synthesized and the reaction was monitored using capillary electrophoresis with UV detection. Then, peroxynitrite was reacted with tyrosine and glutathione under various conditions and the reaction products were detected using the same system. The separations were then transferred to a microchip electrophoresis format with electrochemical detection and the detection conditions were modified to achieve optimal analyte signal. Overall, a greater understanding of the conditions under which these molecules react on chip was attained which will aid in future work detecting these species in cells.

Abstract Text

Keywords: Bioanalytical, Capillary Electrophoresis, Electrochemistry, Electrophoresis

Application Code: Bioanalytical

Methodology Code: Microfluidics/Lab-on-a-Chip
Off-stoichiometry thiol-ene polymers (OSTE) are promising materials for the preparation of microdevices. They present some advantages compared to PDMS based microchips, such as fast polymerization, effective sealing, better wettability, and the possibility of direct surface modification. Here we present the feasibility to graft, in one-step, gold nanoparticles (AuNPs) on OSTE surface by using UV irradiation or incubation with AuNP solution. In the case of surface modification using UV irradiation, localized areas can be prepared, aiming to (i) develop an active surface area to perform Surface Enhanced Raman Scattering (SERS) through the immobilization of AuNPs or (ii) concentrate target compounds present at trace levels in complex matrices (such as biological samples) through the interaction with affinity ligands such as aptamers. We characterized the AuNP-modified surfaces using scanning electron microscopy (JEOL JSM-6360LV, Thermo Electron Corporation, USA equipped with Energy-dispersive X-ray spectroscopy) and SERS performed on a Raman Station 400F spectrometer (Perkin Elmer) equipped with a 785 nm and 250 mW excitation laser. Data were acquired using Spectrum software (Perkin Elmer). Additionally, we tested the chemical resistance of OSTE microchips against background electrolyte solutions commonly used in electrokinetic separations (phosphate or borate buffers containing CTAB or SDS), as well as different organic solvents.
Fluorescence activated cell sorting (FACS) is the most widely used method for isolating fluorescently labeled cells from a heterogeneous population. However, FACS is restricted to sorting viable cells based on extracellular and cell permeable stains, thereby preventing comprehensive sorts involving intracellular biomarkers. Additionally, FACS is inadequate for sorting delicate cell types, like pluripotent stem cells, due to the cell-damaging shear forces it imposes. Microraft arrays are an established and effective cell sorting and cytometry tool that can address the limitations of FACS and similar technologies. Microraft arrays are microwell arrays with each individual microwell containing a magnetic releasable cell culture element (“microraft”). Each microraft cell carrier can be gently released from the array and easily collected with a magnet for assays of their adhered cells. This work involves novel advancements to microraft arrays to allow for automatic sampling of colonies cultured on the microraft array and the magnetic capture of biopsied colony fragments into a replicate microwell array for cell-lethal immunofluorescence staining. By mapping the assayed fragments back to the living original colonies on the microraft array, the original colonies that are identified to express distinct biomarkers can be resampled, and the cells expanded into pure cell lines. To demonstrate the technology’s utility, human peripheral blood cells were reprogrammed into induced pluripotent stem cells (iPSCs) on microraft arrays – a low efficiency transformation (~0.01-1%). The resulting colonies were biopsied and assayed for key pluripotency biomarkers to rapidly identify the fully reprogrammed iPSCs. The NIH provided funding for this work (EY024556).
In vitro Diagnostics aims to provide comfortable experience for patients by taking only a small specimen from blood, urine, or tissues, which contain proteins, DNAs/RNAs, enzymes, small molecules, as well as cells, to serve as specific biomarkers for disease diagnosis, monitoring, and prognosis. Sampling of blood and body fluid using the microfluidic technology presents a simple method for diagnosis with small amount of sample, and with more accuracy, free from complicated handling, pollution and degradation of the sample, and thus the experimental errors. Herein, we will present our recent progress of developing in vitro diagnostic methods based on microfluidics, including microfluidic device for simple distance readout, microfluidic paper-based analytic device for portable detection, and integrated microfluidic device for the processing and readout of Enzyme-linked immunosorbent assay.
Organ-on-Chips have emerged as a novel in vitro technology to model tissue level physiology and response to therapeutics. These devices utilize microfabrication and microfluidic technologies to recapitulate the human in vivo microenvironment. While many different organ chip models have been developed, a challenge remains in the integration of these chips with currently available analysis instrumentation and assays. Previously, we reported a resealable, PDMS-free platform for the interrogation of pancreatic islets. Here, we report a further advanced platform with integrated, non-invasive oxygen sensing. The platform consists of three primary components: (1) a fluidic device, (2) a fluid handling unit, and (3) a user-friendly graphical user interface (GUI). In addition to geometrical features for optimized mass transport, inline debubbling membranes and non-invasive oxygen spot sensors (PreSens, Germany) have been integrated into the roof of the fluidic device (Figure 1A). The fluid handling unit is comprised of a pressure-driven flow controller (Flui gent, France) and a custom-built sampling stage (Figure 1B). The entire platform can be controlled through a single GUI, which allows the user to select from four inlet reservoirs, set the flow rate, and control the sampling stage. To validate the platform’s ability to monitor oxygen levels, local oxygen concentration was recorded during perfusion cultures. Ultimately, our platform demonstrates the ability to utilize existing laboratory instrumentation for on-chip measurements. Future platform development aims to improve durability and usability and apply measurement capabilities to biological phenomenon.
Quantification of alkali metals in water and biological media is imperative for global health and environmental analysis. Unfortunately, accepted methodologies for alkali metal quantification rely on expensive and/or bulky instrumentation. Development of compact lab-on-a-chip (LOAC) technologies utilizing electrochemical methods have also been previously explored but require a power source and a trained operator. We describe an instrument free paper-based microfluidic device modified with nanospheres as an alternative method that circumvents current technological limitations by providing two-phase distance-based quantification for rapid, selective on-site analysis of alkali metals. The chemistry used in our microfluidic devices is demonstrated using an ion selective lipophilic phase containing a potassium selective ionophore (valinomycin), ion-exchanger (sodium tetrakis-[3,5-bis(trifluoromethyl)phenyl]borate) and a pH indicator (chromoionophore I). Incorporation of these compounds into wax-printed or other true “microfluidic” devices has been previously unachievable due to the required usage of organic solvents. We have found, and demonstrate for the first time, that suspension of the lipophilic phase as emulsified nanospheres (178.1 ± 3.2 nm, diameter) in water allows for the addition of the otherwise incompatible lipophilic phase to a wax-printed paper-based device. Quantification of 1–10 mM K⁺ has been demonstrated using a distance-based method that is independent of both sample pH in the range of 6–8 and the presence of interfering ions (Na⁺, Li⁺ and Mg²⁺, 100 mM). We demonstrate that lipophilic phases can be used in paper-based technologies and that alkali metals can be quantified using distance-based disposable microfluidics that do not require electrochemical methods.

Keywords: Environmental/Water, Lab-on-a-Chip/Microfluidics, Quantitative, Sensors
Application Code: Environmental
Methodology Code: Microfluidics/Lab-on-a-Chip
A simple, fast and inexpensive method of TiO2-immobilization on PMMA by drop coating was developed for a new microfluidic UV-LED photocatalytic reactor. Firstly, zigzag micro-channels were engraved in a PMMA chip (15x30x3 mm) with a 100 W CO2 laser set at 20% of power and out of focus, at a speed of 10 mm s⁻¹. A hole (2 mm in diameter) was drilled at each end of micro-channel for input/output of solution. A dispersion of 25 mg TiO2 (P25 Evonik) in 1 mL of ethyl acetate, plus a dispersing additive composed mainly of a phosphoric acid polyester, was applied to the channels. After one hour of drying at room temperature a thin and strongly adhered TiO2-layer remained in the micro-channels. The innovative window sealed on the microfluidic chip was a smartphone screen protector consisting of a thin tempered glass blade with self-adhesive underside (UV-transmittance [greater than] 80% at the LED peak [lambda] of 367 nm). Short silicone tubes were glued to the holes of the PMMA chip and connected to PTFE tubing for solution transport. The UV-radiation source, a 10 W 367 nm UV-LED 3x3 matrix fixed on a heat sink with fan, was operated at half power. The photocatalytic reactor chip was tested with a solution of EDTA, a recalcitrant and poorly biodegradable water pollutant. A 0.1mmol L⁻¹ EDTA solution at pH 6 was pumped through the micro-reactor at various flow rates with the LED ON or OFF. Samples of 100 [micro]L of effluent were spiked with 100 [micro]molL⁻¹ MES solution (internal standard) and injected in a mass spectrometer. The normalized peaks indicated an average decrease of 64% (N = 6 and RSD = 7.76%) of EDTA under irradiation and at flow rate of 96 [micro]L min⁻¹ (94 s residence time in the reactor) Concentrations at lower flow rates were undetectable. The promising first results (with no auxiliary oxidant other than dissolved oxygen) encourage further studies with the new photocatalytic microfluidic reactor with TiO2 coated channels.

**Keywords:** Immobilization, Lab-on-a-Chip/Microfluidics, Sample Preparation, Semiconductor

**Application Code:** Environmental

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

Ultrafast Flow in Multilayer Microfluidic Paper-Based Analytical Devices - FLASH PRESENTATION

Microfluidic paper-based analytical devices (PADs) provide a user-friendly, low-cost method for on-site and point-of-need analyses as an alternative to traditional assays. Despite their widespread adoption, PADs have been previously limited by slow flow rates and the inability to execute automated analytical measurements. Through optical and electrochemical measurements, this study investigates multilayer PADs that generate flow rates >10-fold faster than single layer devices. Previous studies have shown multilayer PADs exhibit faster flow than their single layer counterparts. Herein the sealing, orientation, number of paper layers and gap between the layers in PADs is investigated.

We surveyed these design considerations to find an optimal device architecture. The fluid dynamics were compared to established theories on flow in porous networks. Critically, we demonstrate up to a ~100-fold increase in flow rate (1.5 cm s⁻¹) compared to classical single-layer PADs through device architecture control. These design considerations are being applied to previously reported sequential injection device format where the square wave anodic stripping voltammetry of lead and cadmium to demonstrate the performance and utility of this method.

References

Keywords: Environmental Analysis, Lab-on-a-Chip/Microfluidics, Metals, Stripping Analysis

Application Code: Environmental

Methodology Code: Microfluidics/Lab-on-a-Chip
Microfluidic networks have previously been compared to electrical circuits. The Hagen-Poiseuille law correlates closely with ohms’ law, however outside the basic elements of pressure (voltage), fluidic flow (current), and resistance, the similarities diverge somewhat. Even though complex microfluidic elements (transistors, diodes) have been created, it is inappropriate to assume they will function similarly to their electrical counterparts. Here, we have developed an optical method based on laminar flow to measure relative flow rates, and we have used this method to investigate the switching characteristics of microfluidic transistors and diodes. Our device consists of a network of five channels containing alternating fluorescent dye and buffer which pass through varied microfluidic transistors and converge at an imaging region. Fluorescence imaging of the imaging region coupled with a custom pressure gauge allows extraction of temporal data leading to analysis of the transistor switching states. We demonstrate this method by constructing previously reported (Unger et al., Lab Chip, 2012, 12, 4809-4815.) microfluidic transistors. Three layer devices with sacrificial fill channels were pressurized (from -70 to 210 kPa) with a photocurable acrylate monomer (ditrimethylolpropane tetraacrylate), where photocuring effectively stored the applied pressure. This allowed us to effectively tune the components’ opening threshold pressures. Pressure was applied to the flow channel, and the control (gate) pressure was varied via syringe pump, while control line pressure and optical data were collected simultaneously. As shown in the Figure, transistor switching followed predictable behavior, where break-through pressures correlated well with pre-set fill line pressures. These microfluidic transistor “Q-P” curves (flow rate vs. pressure) represent new information that should be highly useful in designing digital or analog microfluidic circuits in the future.
Acute myeloid leukemia (AML) is the most prevalent form of leukemia among adults. Over 20,000 patients within the United States are diagnosed with AML each year, and each patient has the potential for relapse after five-year remission due to minimal residual disease. Complete remission and patient survival are largely dependent upon the early detection of AML and the age and health of the patient. Therefore, there is a need for a sensitive diagnostic tool that allows medical personnel to detect the presence of myeloblasts, study their behavior and morphology, and examine current and novel cancer treatments with minimal patient stress. Presented in this work is the preliminary design of a microfluidic device capable of detecting myeloid precursor cells (HL60) in blood via CD71 antigen expression. CD71, the Human transferrin receptor, captures cancer cells without a prior knowledge of the cancer type. AML cells were isolated from blood and then tested on the chip for downstream analyses. The capture purity and efficiency are reported as quantitative means to measure the sensitivity and appropriateness of this technique. It is expected that this work will also provide an in-situ platform for chemotherapy and differentiation studies.
We present a droplet-based microfluidic tool for the mass-spectrometric investigation of organic syntheses on a nanoliter scale. The coupling of nano flow systems to mass spectrometry is highly advantageous regarding ionization efficiency and enables fast reaction and analysis times. Lab-on-a-chip applications offer a powerful tool for development and optimization of chemical reactions. Hence, we developed a chip system with seamless connection to electrospray mass spectrometry in order to study chemical reactions. Microfluidic droplet-systems have gained huge importance in biological and chemical research areas and are an outstanding platform for the investigation of transformations at the nanoscale due to efficient mixing. Droplet microfluidics provide fast and controlled change of reaction conditions which is very appealing for screening and optimization of several reaction parameters. A demanding task in lab-on-a-chip applications is the specific and sensitive detection of low-concentrated analytes in small volumes. Herein, we demonstrate a droplet-based chip device for the analysis of heterogeneously catalyzed organic reactions using solid catalyst particles. The developed PDMS chips with integrated ESI-emitter were fabricated in-house utilizing common softlithography techniques. With the developed chip device syntheses can be carried out in segmented flow while the downstream MS detection enables analysis of each individual nanoliter-sized reaction container. Furthermore, controlled encapsulation of solid particles was achieved, enabling the characterization of particulate catalysts. By utilizing the herein presented approach, we demonstrate a proof-of-concept study by means of a catalytic Mannich reaction. This novel tool enables the investigation of reactions heterogeneously catalyzed by discrete amount of catalytic species in unrivalled dimensions of time and space, which is an important step towards the analysis of reactions at the single particle-level.

Keywords: Chemical, Electrospray, Lab-on-a-Chip/Microfluidics, Mass Spectrometry
Application Code: General Interest
Methodology Code: Microfluidics/Lab-on-a-Chip
Preterm birth (PTB) affects 1 in 9 children born in the United States. Our research focuses on developing 3D printed microfluidic devices for the quick and inexpensive analysis of a panel of PTB biomarkers found in maternal blood serum. We have 3D printed a 45 μm by 40 μm microfluidic channel with a unique porous polymer monolith exposure window. In addition, conditions for the polymerization of affinity monoliths in these 3D printed devices have been optimized. A labeled PTB biomarker, ferritin, is electrophoresed through the monolith and detected by laser induced fluorescence. The ability of the affinity monolith to retain and elute labeled ferritin is evaluated in order to determine the best conditions for extracting ferritin. Demonstration of the extraction of ferritin is a key step in the development of an integrated 3D printed microfluidic device that can be used to predict PTB risk.

Keywords: Chromatography, Electrophoresis, Lab-on-a-Chip/Microfluidics, Sample Preparation
Application Code: Bioanalytical
Methodology Code: Microfluidics/Lab-on-a-Chip
Here we demonstrate the separation of neutral species from human blood plasma by ion concentration polarization (ICP) at a branched micro-/nano- fluidic junction. This separation can be employed to source fluid from blood that can be utilized downstream, as patient-derived dialysate, to accept metabolic waste during hemodialysis. We demonstrate device configurations that avoid damage to blood components, such as protein denaturation and device biofouling, resulting in increased device lifetime. To further improve the device performance, we have investigated two means of driving ICP - employing either an ion selective membrane or a bipolar electrode (BPE). Our results provide a quantitative comparison of these systems and describe the contribution of experimental parameters to separation efficiency.
We report initial results on a new method of separating blood plasma from whole blood. The device works using dielectrophoresis (DEP) running at low frequencies to repel all cells from areas of high electrical field gradient. The electrical voltage is applied using liquid electrodes behind a thin insulating wall (polydimethylsiloxane (PDMS) membrane) to create a uniform electrical field across the entire height of the device. The wall also isolates the electrodes to avoid biofouling, which can occur at a metal electrode. Local electrical field gradients are established by an array of angled oblong pillars. The pillars are offset such that the electrical field gradient is established between them in the x-direction to create virtual walls that will discourage entry by cells while leaving fluid flow unhindered. We anticipate that this approach will leave both the plasma and cell-containing fractions undamaged. This feature is essential because we intend to utilize this DEP-based blood fractionation as the first step in a new form of hemodialysis being developed in our lab.

Keywords: Bioanalytical, Lab-on-a-Chip/Microfluidics, Separation Sciences
Application Code: Bioanalytical
Methodology Code: Microfluidics/Lab-on-a-Chip
Efforts to reduce deaths caused by sepsis would be greatly advanced by the ability to rapidly detect the presence of bacterial drug resistance genes in a blood sample. A novel strategy for probing drug resistance genes is to use a system capable of detecting each molecule individually. A key technical challenge of this approach is ensuring that the captured genes provide an appropriate signal to noise ratio. The focus of our research has been to develop sequence-specific capture of nucleic acids suitable for single molecule detection.

To capture DNA segments that are long enough to accept multiple labels for easy detection, we have utilized magnetic beads modified with a 25 mer ssDNA complementary to the target sequence. We have been able to capture 500 base pair PCR amplicons of clinical isolates from a 100 µL sample in less than 15 minutes. We have also been able to integrate these methods into a single channel, pressure driven microfluidic system. The system was capable of concentrating 10 femtomoles of target DNA to a level detectable by laser-induced fluorescence. These results represent an important step in the process of creating viable technology for the rapid detection of drug resistance. Such technology will be critical in the development of devices that can be used in the diagnosis of sepsis.

Keywords: Fluorescence, Lab-on-a-Chip/Microfluidics, Microscopy
Application Code: Bioanalytical
Methodology Code: Microfluidics/Lab-on-a-Chip
Enzyme assays have been traditionally performed with complicated sample preparation on bulky spectroscopic instruments. Point of care analysis of enzyme activity usually involves careful sample treatment and storage. We aim to cope with these difficulties by developing an online sample preparation isoelectric focusing device. Isoelectric Focusing (IEF) has been a regular method in peptide/protein separations, offering high sensitivity and great resolving power. IEF provides a local pH gradient, which causes peptides of different isoelectric points to carry charges and get separated under electric field. As a demonstration, trypsin is used as the target enzyme. Fluorescently tagged peptide substrates, linked by sulfur double bond on magnetic nanoparticles, mingle and incubate with trypsin containing samples. After trypsin digestion, these magnetic particles are moved to an area where all linked peptides are stripped off. These peptide substrates, cleaved or uncleaved by the trypsin, are separated and focused inside a microfluidic channel based on their isoelectric points. Fluorescent signals of separated peptide fragments are read out by scanning laser induced fluorescence detection setup across the microfluidic channel.

**Abstract Text**

**Keywords:** Bioanalytical, Electrophoresis, Lab-on-a-Chip/Microfluidics, Sample Handling/Automation

**Application Code:** Bioanalytical

**Methodology Code:** Microfluidics/Lab-on-a-Chip
Enzyme linked Immunosorbent Assay (ELISA) has been the gold standard plate based protein detection technique for the last few decades. Fully automated ELISA protein detection devices are available with complex engineering, robotics, aspirators and fluid control at high cost and size. Here, we introduced pipette tip based ELISA as a next generation tool with better sensitivity, shorter incubation time and less sample and reagent volumes than traditional ELISA. Integration of this technique in fully automated devices would eliminate the need for complex engineering and fluidic control with anticipated reduction in device size and cost. We applied this technique in detection of four cancer protein biomarkers with detection limits in femtogram range and a wider dynamic range compared to plate based ELISA. Recoveries from spiked human serum samples were from 92% to 108% with standard deviation less than 5%. We also demonstrated the ability to utilize either colorimetry with tetramethylbenzidine (TMB) or chemiluminescence with luminol as enzyme substrates.
Creating validated genetically modified cell lines for disease modeling studies is often cost prohibitive because the current state of the art is labor intensive and experimentally challenging. Here, we describe the implementation of a novel silicon microwell device for simultaneous, high-throughput PCR validation of >100 clonal colonies. The device design greatly reduces pipetting steps and significantly decreases time and reagent costs relative to traditional sib-selection and validation. Seeding cells at low densities on custom microrafts replaces the use of large volume petri dishes and repetitive dilutions. Clonal colonies are verified by microscopy and validated by microfluidic PCR reactions, thereby decreasing the preparation time necessary for creating useful disease model systems. Within one week of seeding, clonal colonies are transferred from the microraft array into a silicon PCR chip consisting of an array of >1,000 etched microwells. The microwells (50 nL) are then filled with a PCR mix before isolating them with a sealing oil. Cells are heat-lysed, and the array is temperature cycled using a custom thermoelectric stage. A modified microscope is used to measure the fluorescence intensity of each well as product is generated in each PCR cycle. Proof of concept studies have shown successful transfer of >90% of cell colony carriers and amplification of extracted genomic DNA within the silicon microwells. Further work demonstrated positive amplification with 20 to 50 whole cells per well. This high-throughput analysis platform will significantly reduce the time, cost, and technical skill necessary to generate validated stem cell lines for modeling genetic disorders.

Funding is provided by NIH grant # 5-R01-EY024556-01-02

Abstract Text
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Funding is provided by NIH grant # 5-R01-EY024556-01-02

Keywords: Bioanalytical, Fluorescence, High Throughput Chemical Analysis, Lab-on-a-Chip/Microfluidics
Application Code: Bioanalytical
Methodology Code: Microfluidics/Lab-on-a-Chip
Sepsis is 11th cause of death in hospitals in the US. Currently, the “gold standard” for sepsis diagnosis is the culture of sputum, urine, and/or blood for suspicious pathogens, which typically takes 2-14 days. We have demonstrated a rapid and early sepsis detection method using CD64+ cell capture in a microfluidic affinity chip. This method reports total on-chip cell counts as a label free and quantitative detection for sepsis. Both a laboratory CD64 expression model and sepsis patient samples were used to validate the method. Flow cytometry analysis showed that on-chip cell capture had a linear relationship with CD64 expression. The Sepsis Chip detected an increase in upregulated neutrophil-like cells when the upregulated cell population is as low as 10% of total cells spiked into commercial aseptic blood samples. In a proof of concept, blood samples obtained from sepsis patients within 24 hours of diagnosis were tested on the chip to further validate its performance. The positive detection of CD64+ cell can be as early as 4 h in model and within 24 h for real patient samples including sample processing time. In addition, second blood draws at 48 hours from the same patients undergoing antibiotic treatment showed decreased CD64+ cell counts, indicating our approach is suitable to track recovery as well. Our method has proven to be sensitive, accurate, rapid, and cost-effective. Since our chips measure immune system response to sepsis, rather than the pathogens that trigger immune response, they are particularly well suited to situations when the blood culture is negative but sepsis symptoms are present. In conclusion, this approach is rapid, can detect neutrophil activation early, and can be operated with any simple microscope at minimum cost.
Acute febrile illnesses are responsible for significant mortality and morbidity in developing settings. Current diagnostic gold standards are often impractical for use in these settings due to the lack of infrastructure and trained personnel. In this work, we are developing the FeverPhone (Fig. 1), a mobile platform that enables rapid point-of-care multiplexed analyte detection.

Recent efforts are focused on detecting and differentiating between dengue and chikungunya IgM/IgG, which is of clinical importance as NSAIDs used to alleviate the symptoms of chikungunya can increase the risk of hemorrhage in dengue patients. As shown in Fig. 2, the architecture of the lateral flow assay which forms the key element of the diagnostic is as follows: a sample filtration pad, a conjugate pad, a nitrocellulose membrane, and a wicking pad. Red (dengue) and blue (chikungunya) 400nm latex particles were functionalized with viral proteins through NHS-esters-based covalent conjugation chemistry. Functionalized particles were subsequently able to bind to disease-specific antibodies present in the sample. These antibody-latex complexes then contributed to the development of colorimetric signals which can be captured by the reader. We achieved low limits of detection (~1ng) with an assay design that is cost effective and at the same time better suited for use with clinical sample matrices.

Funding acknowledgement: NIH grant R01EB021331

Keywords: Biomedical, Immunoassay, Lab-on-a-Chip/Microfluidics, Portable Instruments

Application Code: Biomedical

Methodology Code: Microfluidics/Lab-on-a-Chip
Preterm birth (PTB) is the leading cause of neurological disabilities and infant mortality. Over one million infants died in 2013 from PTB related complications. Esplin, et al. found that PTB risk can be determined with 87% selectivity and 81% specificity through nine maternal blood serum biomarkers. One of these biomarkers is the thrombin-antithrombin III complex (TAT). TAT cannot be purchased so it must be synthesized in the lab to be analyzed. We have formed TAT in buffer and analyzed the results by dot blot test, capillary electrophoresis, and microchip electrophoresis. Once synthesized, we were able to separate TAT from six other PTB biomarkers using microchip electrophoresis. We are presently working to integrate this separation with upstream microfluidic sample preparation steps for the diagnosis of PTB risk.

Reference

Acknowledgement
We thank the National Institutes of Health (R01 EB006124) for funding this work.
Extracellular vesicles (EVs) have emerged as important mediators for intercellular communications involved in many pathophysiological conditions, such as cancer progression and metastasis. EVs are membrane-enclosed vesicles of endocytic origin and contain proteins and nucleic acids. They are secreted by almost all types of cells and enter the circulation. Recently, EV-associated messenger RNA (mRNA) and microRNA (miRNA) have attracted considerable attention as biomarkers for cancer detection. Capturing EVs from body fluids and identifying the encapsulated mRNA/miRNA targets has become a promising approach to achieving non-invasive cancer diagnosis as well as monitoring of treatment response.

We present here a facile and yet powerful signal-amplifiable biochip based on lipid-polymer hybrid nanoparticles containing catalyzed hairpin DNA circuit (LPHN-CHDC) that can enhance sensitivity and specificity in identifying extracellular vesicle (EV)-associated RNA targets important for non-invasive early-stage cancer detection. The core-shell-corona structured lipid-polymer hybrid nanoparticles (LPHN) provides unique advantages over commonly used onion-like lipoplex nanoparticles (LNs) for encapsulating molecular beacon (MB) or catalyzed hairpin DNA circuit (CHDC) for hybridization with EV-associated RNAs. CHDC exhibits superior performance to conventional MB for achieving effective imaging and enzyme-free signal amplification of target RNAs in-situ. LPHN-CHDC biochip with signal-amplification capability could selectively and sensitively identify low-expression glypican-1 mRNA in serum EVs, distinguishing patients with early- and late-stage pancreatic cancer from healthy donors and patients with benign pancreatic disease. ROC curve of LPHN-CHDC showed an AUC of 1.0 in PDAC patients of stage I-IV compared to healthy donors and BPD patients, with a sensitivity and specificity of 100%, highlighting their clinical potential in early cancer diagnosis and therapeutic monitoring.
We report a digital droplet microfluidic approach for the tunable synthesis of silica-encapsulated gold nanoparticles (si@AuNPs) for optical imaging using surface enhanced Raman Spectroscopy (SERS). SERS is a potentially powerful analytical technique, especially in aiding medical diagnosis. However, there remain key challenges in the translation of this technique to routine clinical use. The requirement for particle monodispersity, surface chemistry, shape and size control are essential in translating NPs into medical and clinical applications, thus the need for controllable technology. Droplet microfluidics offer precise control over the chemical and physical properties at all points in the reactor, resulting in predictable, consistent and uniform reaction conditions for the synthesis of NPs. Silica encapsulated gold nanoparticles (Si@AuNPs) offer enhanced Raman scattering properties compared to AuNPs. We demonstrate that by simply adjusting parameters of the digital droplet microfluidic device such as flow rate, tunable and reproducible NPs of specific size and Si encapsulation thicknesses could be synthesized. By varying parameters in the synthetic process and characterizing the NPs with transmission electron microscopy (TEM) and energy dispersive x-ray spectroscopy (EDS), we have optimized the process to achieve desired AuNP size and Si encapsulation thickness. Synthesized particles were used for cancer cell imaging, demonstrating the tunability and performance required for the next generation of imaging.
Herbal Nanomedicine is the well proven medical application of nanotechnology. Nanomedicine ranges from the medical applications of nanomaterials, to nanobiosensors. Current problems for Herbal nanomedicine involve understanding the issues related to poor bioavailability of herbal compounds, toxicity and environmental impact of nanoscale materials. Use of Nanotechnology in synthesis Herbal nanomedicine refers to approaches, formulations, technologies, and systems for transporting a Herbal compound in the body as needed to safely achieve its desired therapeutic effect. Herbal nanoformulations modify drug release profile, absorption, distribution and elimination for the benefit of improving product efficacy and safety, as well as patient convenience and compliance. Many medications using plant secondary metabolite or natural compound such as curcumin and Qurectein, and Thymoquinone based drugs, in general may not be delivered using normal routes because they might be susceptible to enzymatic degradation or cannot be absorbed into the systemic circulation efficiently due to poor solubility in water, molecular size and charge issues to be therapeutically effective. For this reason many herbal medicine have to be delivered by using the concept of nanomedicine. Current efforts in the area of drug Herbal Nanomedicine include the development of targeted delivery systems in which the Herbal molecule is only active in the target area of the body (for example, in liver tissues) and Characterisation of such formulation using TEM, SEM and DLS. Out of all such techniques Transmission Electron Microscope is the well accepted technique amongst the scientific community to characterise the metallic as well as polymeric nanoparticles. Transmission Electron Microscope is considered to be the most authentic tool to determine the shape of nanorods and nanowires as well their sizes.
In this study, we have developed a simple method to synthesize gold nanocomposites with photoluminescent gold nanodots (Au NDs) via etching of (11-mercaptoundecyl)-N,N,N-trimethylammonium bromide (11-MUTAB) on gold nanoparticle and gold nanostars (Au NS). The as-prepared 11-MUTAB–Au NDs/Au NSs can inhibit not only normal pathogens [i]Escherichia coli[/i] ([i]E. coli[/i]), [i]Proteus vulgaris[/i] ([i]P. vulgaris[/i]), [i]Salmonella enterica[/i] ([i]S. enterica[/i]), [i]Staphylococcus aureus[/i] ([i]S. aureus[/i]), but also multidrug-resistant bacteria [Methicillin-resistant [i]Staphylococcus aureus[/i] (MRSA) and [i]Pseudomonas aeruginosa[/i] ([i]P. aeruginosa[/i])]. The antimicrobial activity of 11-MUTAB–Au NDs/Au NSs is independent of quaternary ammonium compound (11-MUTAB) and controlled by the synergistic effect of 11-MUTAB and Au NDs on the broken of bacterial membrane. Moreover, Au NSs have enhanced surface plasmon resonance and could be photoexcited to induce multiple radicals from 11-MUTAB–Au NDs. The minimal inhibitory concentration values of 11-MUTAB–Au NDs/Au NSs under irradiation are lower than without irradiation. [i]In vitro[/i] cytotoxicity, hemolysis, hemagglutination, genotoxicity and oxidative stress, and in vivo morphologic and physiologic cornea change evaluations show the good biocompatibility of 11-MUTAB–Au NDs/Au NSs. This study suggests that photoexcited 11-MUTAB–Au NDs/Au NSs could be used in the study of the effect of redox states, and lead to antimicrobial candidate for preclinical applications.
Halloysite is a clay that naturally forms nanotubes (HNTs) and has recently come to attention in biomedical applications. As an effective drug carrier and intrinsic anti-inflammatory, HNTs can be used to deliver anti-inflammatory drugs in conjunction with a tissue engineering strategy for the potential treatment of SCI. HNTs are loaded with sulindac, an NSAID, and electrospun with gelatin in their unloaded and loaded forms. The electrospun webs are characterized through tensile testing, SEM, EDS, FTIR, and DSC and their morphologies and mechanical properties analysed. Addition of HNT increased the strength of gelatin fibers while maintaining elasticity and the 3 wt% HNT-gelatin scaffold displayed optimal properties. Fiber alignment also decreased with increasing HNT percentage, and the drug loaded fibers did not generally display differing mechanical properties or morphology. The 3 wt% HNT scaffold is recommended for future drug delivery and tissue engineering studies.

**Keywords:** Bioanalytical, Biomedical, FTIR, Thermal Desorption

**Application Code:** Nanotechnology

**Methodology Code:** Microscopy
As Nanotechnology continues to gain importance in both fundamental and applied sciences, the need to measure size has grown with it. Of the various techniques available to characterize nanoparticle size (such as atomic force AFM, transmission electron TEM, or scanning electron microscopy SEM, and also small angle X-ray scattering SAXS), we concentrate on dynamic light scattering DLS in this study.

The method itself is a rapid, cuvette-based ensemble measurement that extracts information about the diffusion coefficient of the nanoparticles from correlation analysis of the underlying signal fluctuations. DLS is also known as photon correlation spectroscopy PCS or quasi-elastic light scattering QELS. Due to the nature of the technique, the data will be “low resolution” and contain an inherent polydispersity of even perfect standard materials.

We investigate this polydispersity as well as the other key characteristics of the analysis (z-average cumulant size, intensity distribution, volume distribution) at the practical example of the Nanoparticle reference standard from the National Institute of Standards & Technology. The NIST Reference Material RM8011 contains citrate-stabilized colloidal gold nanoparticles of nominal 10nm diameter.

The experimentally determined standard deviations of the key measurement parameters such as mean size and overall polydispersity may serve to set expectations and as guidance for researchers sizing non-standard samples of unknown nanoparticles that tend to be more polydisperse and show larger statistical variances. These results set a bench mark reference point for a ‘best and smallest in class’ reference material near 10nm diameter.

Keywords:  Light Scattering, Nanotechnology, Particle Size and Distribution, Reference Material
Application Code:  Nanotechnology
Methodology Code:  Physical Measurements
Biomimetics is a new technical field in which the imitation of the functions and structures of biological organisms is applied to engineering developments and manufacturing. The structural color pellets which were made of biomimetic core-shell particles (core material : polystyrene particle) with melanin-like polydopamine (PDA) shell layers were measured with a UV-Visible spectrophotometer and an atomic force microscope (AFM) to investigate the relationship between the color and the particle. In the reflection spectra of the pellets, the reflection peaks shifted to longer wavelength as the thickness of PDA shell layer increased because the increase of the thickness enhanced the refractive index of the biomimetic core-shell particles. Their AFM measurements results revealed that particles with thick PDA shell layers composed amorphous structures to form the rough particle surface while non-coated polystyrene particles and those with thin PDA shell layers composed colloidal crystal structures. The structural difference between them caused the change of the structural color. The structural color of the pellets depends on the size, blackness, refractive index, and arrangement of the biomimetic core-shell particles. In this work we demonstrate their spectroscopic and structural study. The following figures are shown to understand the abstract written above.
The vitamin E derivative, alpha-tocopheryl phosphate (TP), occurs in small amounts in plasma and animal tissues. Low amounts of TP are also present in foods but the bioavailability as an intact molecule is very low, since most of it is hydrolyzed. So far no efficient mechanism of intact cellular TP uptake and tissue distribution has been identified when compared to vitamin E (alpha-tocopherol, T). Since TP has been reported to be more potent in regulating signal transduction and gene expression than T (e.g. on atherosclerosis, inflammation), a more efficient system to deliver intact TP to cells in tissues may be useful. Here we characterize the formation of complexes of TP with the nanocarrier cyclodextrin (CD) by thin layer chromatography (TLC), electron microscopy (SEM and TEM), and dynamic light scattering (DLS). TP/CD formed soluble complexes in a 1 to 2 ratio and generated a homogenous white colloidal emulsion that stayed in homogenous suspension for weeks. The TP/CD complex inhibited proliferation of THP-1 monocytes and CaCo2 colon carcinoma cells slightly stronger than TP. When compared to CD, the complex of TP/CD extracted cholesterol from cellular membrane of cultured THP-1 monocytes and HT29 intestinal epithelial cells with higher efficiencies and was associated with delivery of TP to these cells. The TP/CD complex induced VEGF promoter activity in THP-1 and HEK293 cells with slightly higher efficiency than TP. TP/CD assembled to higher order complexes with three types of modified starches and chitosan. These higher complexes had a comparable activity in the above assays and therefore may be useful as protective vehicle for oral, dermal or ocular delivery or as deposit enhancer in mucosa for delivery across epithelia.
Our work is based on the development of nanomaterials from pharmaceutical drugs, as a systematic strategy for delivering therapeutic agents to target the body receptors. The investigated nanomaterials, composed purely of drug molecules (nanodrugs), exhibit intrinsic therapeutic properties, and are designed in absence of a delivery agent. The administration of these nanodrugs can induce a highly efficient biological effect by delivering multiple therapeutic agents per nanoparticle. Furthermore, the formulation of nanomaterials from drugs offers a bioavailability for an extended time in comparison to their bulk counterparts. Herein, we applied customized syntheses of those nanodrugs in various therapeutic applications. For example, drug nanoformulations with cooling properties were prepared using vanilloid compounds, for application in therapeutic hypothermia. Those formulations will be administered to secure neuroprotection in the case of acute traumatic injuries. Moreover, several formulations, derived from an antiviral drug, were prepared for targeting Zika virus. The prepared formulations were made of self-assembled nanovesicles, potentially offering a significant permeation ability through biological membranes. The formation of size-controlled nanodrugs was performed using bottom-up approaches, assisted with ultrasound and soft templates. Transmission electron microscopy and dynamic light scattering showed the formation of various structures of nanoscale materials, with most formulations being monodisperse. Zeta potential measurements indicated a high stability of the nanosuspensions, obtained upon optimization of the synthetic procedure. Current work focuses on developing biological assays suitable for testing the nanoparticles in each type of therapy. Overall, this research can contribute to the advancement of nanotechnologies in the fields of materials design and targeted therapies, which should have an impact in treatment of injuries and disease states.

Keywords: Materials Characterization, Nanotechnology, Particle Size and Distribution, Wet Chemical Methods
Application Code: Nanotechnology
Methodology Code: Physical Measurements
Atmospheric aerosol particles of sizes smaller than 2.5 microns are particularly harmful to human health. Due to their complex generation conditions, the composition and organization of the aerosol particles are difficult to predict. Joint chemical and mechanical characterization are much needed to decipher their formation processes. In this presentation, we present the utilization of a recently developed technique, the peak force infrared (PFIR) microscopy on individual atmospheric aerosol particles with sizes smaller than 500 nm. The PFIR technique combines the action-based infrared microscopy with the peak force tapping mode of atomic force microscopy. The PFIR microscopy allows sub 10 nm spatial resolution infrared imaging, broadband infrared spectroscopy, and mechanical mapping on the same sample. With the PFIR, the inhomogeneities of the aerosol particles are revealed that are indicative of the formation mechanism of aerosols.

Based on the spectroscopic and mechanical signature, the primary organic aerosols and secondary organic aerosols can be distinguished based on the concentrations of carbonyl bonds as well as surface adhesions. Besides the organic aerosols, the PFIR microscopy is capable of characterizing inorganic aerosols such as ammonium sulfate, a frequent present compound in atmospheric aerosols. The attached figure shows the topography (a), modulus (b), adhesion (c), infrared imaging at 1420 cm⁻¹ (d), as well as an infrared spectrum on an aerosol particle (e). The nanoscale characterizations of individual aerosol particles are expected to be complementary to the current popular ensemble characterization methods for the understanding of the formation and transformation of sub-2.5-micron atmospheric aerosol particles.

Keywords: Aerosols/Particulates, Atomic Force Microscopy (AFM), Infrared and Raman, Nanotechnology
Application Code: Environmental
Methodology Code: New Method
Nanotechnology - Physical Measurements

Multiple Light Scattering as a Tool to Monitor Size Evolution of Nanoparticles During Wet Milling, Comparison with Laser Diffraction and DLS

Nano suspensions or emulsions are widely used in the industry but their real dispersion state remains unknown or not well characterized in their native and concentrated form. Indeed, it is well known that aggregation and agglomeration may exist in concentrated regime. A technique of Static Multiple Light Scattering (SMLS) is proposed to measure mean particles size in a large range of concentration between 0.0001 and 95%, for sizes between 10 nm and 100 µm by Turbiscan LAB technology. Turbiscan consists in sending a light source (880nm) and acquiring backscattered and transmitted signal. The signal intensity enables to measure directly the mean spherical equivalent diameter \(d\), knowing refractive index of continuous \(n_f\) and dispersed phase \(n_p\) and the particles concentration \(\phi\) according to the Mie theory:

\[
d = f(BS \text{ (or } T), \phi, n_p, n_f)
\]

with BS for Backscattering Intensity and T for Transmission Intensity.

This technique has the advantage to measure in one click, without sample preparation or dilution, the mean particles size and so the dispersibility efficiency particularly for concentrated suspensions. Other optical techniques such as DLS, PTA or laser diffraction can perform this measurement but only at a very high dilution which denatures the agglomerates and give an erroneous size of the native particles.

In this work, we present a complete study of size measurement of silica particles exposed to wet milling and going from micro to nanometer nanometers. This size is measured with SMLS and compared with DLS and Laser Diffraction.

We propose also to present comparison of SMLS with SEM/TEM microscopy size measurement.

Keywords: Characterization, Light Scattering, Materials Characterization

Application Code: Nanotechnology

Methodology Code: Physical Measurements
The brain’s metabolic demands require an intricate network of blood vessels to carry energy – mostly in the form of glucose and oxygen – to brain cells, including astrocytes and neurons. The prevailing hypothesis is that increased neuronal activity leads to high metabolic demand of oxygen, causing elevated blood flow, increasing oxyhemoglobin, and decreasing deoxyhemoglobin concentrations. This dynamic event is known as neurovascular coupling (NVC) and understanding its basic mechanism is crucial to correctly correlate neuronal activities and vascular responses. Nonlinear optical imaging is an attractive method for such an application due to its intrinsic 3D imaging capabilities and high spatial resolution. Routinely, two-photon excited fluorescence (TPF) is employed to visualize calcium dynamics, a hallmark of neuronal spiking activity, through the use of Ca2+ indicators and voltage sensitive dyes. However, real-time quantification of local blood oxygenation in neighboring blood vessels has not been demonstrated, a pivotal step in understanding the underlying mechanism of NVC. In this work, we present real-time in vivo quantification of blood oxygenation in mouse brain vasculature via two-photon absorption (TPA). Additionally, we couple simultaneous quantification of blood oxygen concentration with TPF of calcium dynamics to further elucidate the mechanism of NVC at superior spatial and temporal resolution than previously reported.
In order to understand membrane transport and thereby develop suitable membranes for protection and separation, there are a few characteristics of the membrane and the diffusing constituents that must be known. These include the molecular states of the diffusing components, their diffusion coefficients and the membrane selectivity. The fundamental physical property required for designing and optimizing polymers used as barriers and membranes or in polymer processing operations is the diffusion coefficient. For many years, polymer/penetrant mutual diffusion coefficients have been measured using dip and weigh, GC and vapor sorption experiments. Neither of these methods allows the transport of two or more components to be monitored.

In this work, the transport of commercial solvents through polymer membranes by using time resolved FT-IR-ATR is studied. Polymer samples were deposited onto a horizontal IRE crystal and a challenge liquid mixture consisting of one or two penetrants were allowed to flow over the sample. The IR spectra at the polymer/crystal interface were monitored using conventional GC software allowing the kinetics of the transport process to be evaluated.

Keywords: FTIR, Material Science, Membrane, Polymers & Plastics
Application Code: Polymers and Plastics
Methodology Code: Vibrational Spectroscopy
**Abstract Text**

Single- and few-layer transition metal dichalcogenides (TMDCs) are mainly characterized by layer thickness, chemical composition, and exciton contribution using atomic force microscopy (AFM), Raman, and photoluminescence (PL) techniques, respectively. Raman and PL measurements necessitate that the sample be exposed to laser radiation for various time periods. What is the impact of laser power and time on the WS$_2$? This presentation will summarize recent research exploring the role of laser power and exposure time on the observed PL emission and topography from WS$_2$ on Au.

**Keywords:** Atomic Force Microscopy (AFM), Imaging, Luminescence, Raman Spectroscopy

**Application Code:** Material Science

**Methodology Code:** Vibrational Spectroscopy
Submultiple Data Collection to Explore Spectroscopic Instrument Instabilities Shows that Much of the "Noise" Is Not Stochastic

As has long been understood, the noise on a spectrometric signal can be reduced by averaging over time, and the averaged noise is expected to decrease as $t^{1/2}$, the square root of the data collection time. However, with contemporary capability for fast data collection and storage, we can retain and access a great deal more information about the signal train than its average over time. During the same collection time, we can record the signal averaged over much shorter, equal, fixed periods. This is, then, the set of signals over submultiples of the total collection time. With a sufficiently large set of submultiples, the distribution of the signal's fluctuations over the submultiple periods of the data stream can be acquired at each wavelength (or frequency). From the autocorrelations of submultiple sets, we find only some fraction of these fluctuations consist of stochastic noise. Part of the fluctuations are what we call 'fast drift', which is defined as drift over a time shorter than the complete measurement period of the average spectrum. In effect, what is usually assumed to be stochastic noise has a significant component of fast drift due to changes of conditions in the spectroscopic system.

In addition, we show that the extreme values of the fluctuation of the signals are usually not balanced (equal magnitudes, equal probabilities) on either side of the mean or median without an inconveniently long measurement time; the data is almost inevitably biased. In other words, the unbalanced data is collected in an unbalanced manner around the mean, and so the median provides a better measure of the true spectrum. As is shown here, by using the medians of these distributions, the signal-to-noise of the spectrum can be increased and sampling bias reduced. The effect of this submultiple median (SMM) data treatment is demonstrated for infrared, CD, and Raman spectrometry.

Keywords: Infrared and Raman, Instrumentation, Spectrophotometry
Application Code: General Interest
Methodology Code: New Method
The world faces many emergent medical threats and healthcare challenges. I will discuss how precision medicine may offer more efficient and effective healthcare solutions in the 21st century. In an effort to achieve that objective, individual patient descriptions are required at the level of the genome, proteome, metabolome, or a combination of these aided by state-of-the-art technologies. High resolution analytical technologies and sophisticated computational modeling can create both new diagnostics and prognostics of disease ultimately leading to disease prevention in the future.

Keywords: Biomedical, Drug Discovery, Genomics, Proteomics
Application Code: Biomedical
Methodology Code: Education/Teaching
The column has always played a pivotal role in the capability of LC to solve “real world” problems. From the early days of large particle superficially porous packings (SPP) to the eventual decades of domination of 5 micron 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 an elevated level of contribution to the analytical laboratory. We will briefly look at the history of particle development and then focus in the latest packing materials: sub-2 micron porous particles, small particle SPP and monoliths. Without getting too involved in the theory, we will discuss the pros and cons of these latest columns and give some practical advice on which approach might fit better in your own laboratory. We will discuss how these columns work with modern HPLC/UHPLC instrumentation and what can be done to get the best performance, especially with respect to band broadening, pressure capability and detector interfacing. Advances in stationary phase technology in various LC modes will be highlighted and may in the long run provide even better resolution than particle size developments alone. The concluding part of this talk will briefly look at possible future directions for column technology such as engineered (ordered) columns via micelle templating with pseudomorphic transformation, pillar arrays and the possible application of 3D printing techniques. The presentation should provide a better understanding of modern HPLC/UHPLC columns, their features, and their advantages and disadvantages.

Keywords: Chromatography, HPLC, HPLC Columns, Liquid Chromatography
Application Code: Other
Methodology Code: Liquid Chromatography
Large molecules are vitally important in research and industry, and HPLC or LC-MS are becoming preferred analytical methods. Separation of large molecules requires both knowledge of molecular size and intelligent selection of column particles to achieve good results. The dilemma is that particles must be porous or superficially-porous with high surface-area and stationary phase loading to create a viable, two-phase distribution system; however, pores must also be large enough to grant all solutes easy access to stationary phase. Commercial silica columns typically have a broad pore-distribution that accepts and separates a range of different solute sizes. When size varies significantly within the sample, performance loss may be seen as diameters of larger solutes begin to approach diameters of smaller pores. For good performance, method development requires a careful match between column pore-size range and the range of solute sizes within the sample. Ideally, HPLC particles should permit all solutes to enter pores and diffuse freely without interference from the particle walls. This paper will review what is known about pores, how they compare in size to solutes, how to make early column selection decisions to maximize chances for good separation, and how to solve problems if poor performance is observed. Available data suggests that the diameter of target molecules should be no larger than 10% of the mean pore diameter in order to minimize pore-crowding that can destroy retention, efficiency and resolution. New particle designs for improved separation of larger molecules will be described.

Keywords: Bioanalytical, HPLC, HPLC Columns, Liquid Chromatography/Mass Spectroscopy
Application Code: General Interest
Methodology Code: Liquid Chromatography/Mass Spectrometry
HPLC columns have come a long way from the late 1960s and early 1970s when users packed their own 1 m long columns with hand-sieved, crushed silica, often coated with a non-polar liquid phase. Today most HPLC columns are commercially packed in lengths of 50-150 mm and packed with <2 – 5 micron spherical particles, usually with a stationary phase bonded to the surface. Many changes in technology over the past 50 years have made these columns much more reliable than their ancestors, but they are not without problems.

This talk will review some of the problems from the early days of HPLC and how they were addressed, both by the user in the lab and by column manufacturers as techniques were improved. It will also cite some of the most common problems users encounter with columns today and share some procedures to help prevent or minimize such problems.

Keywords: Chromatography, HPLC, HPLC Columns, Liquid Chromatography
Application Code: General Interest
Methodology Code: Liquid Chromatography
Recently, a series of high efficiency HPLC chiral stationary phases based on macrocyclic glycopeptide and cyclofructan chiral selectors have been developed. The improved efficiencies observed in HPLC were afforded by the use of i) superficially porous particles (SPPs) or ii) sub-2um fully porous particles (FPPs) with narrow particle size distributions (NPSD). Both the SPP and NPSD chiral columns exhibited over 200,000 plates per meter when tested in HPLC. For example, when using a cyclofructan based CSP, at a flow rate of 3.0 ml/min, the number of plates on column afforded by the SPP column was ~ 7x greater than the number of plates on column (same length) obtained when using a commercial 5 µm FPP based column. Under constant retention conditions, it was demonstrated that a SPP based CSP greatly improved resolution compared to commercial columns. Given their high efficiencies and relatively low back pressures, columns containing these SPP particles were particularly advantageous for ultra-fast “chiral” separations in the 4 to 40 seconds range. Further, highly efficient chiral phases were produced using novel, sub-2 µm, NPSD, Titan silica particles. The UHPLC chromatographic performance was compared to state of the art 5 µm commercial columns, as well as, 1.7 µm chiral phases made in-house. The latter comparison gives a clear picture of the true advantages of the Titan material over other sub-2 µm silica. The high efficiencies of these CSPs allowed for the use of short columns and an additional means to produce ultra-fast enantiomeric separations. With either novel column technology, the practice of ultra-fast chiral LC often produces interesting and unusual consequences that must be recognized, dealt with, and/or properly understood for optimal performance.

Disclosure: Zachary S. Breitbach is a current employee of AbbVie Inc. Data present employment.

Keywords: Chiral Separations, Chromatography, HPLC, SFC
Application Code: General Interest
Methodology Code: Liquid Chromatography
Room-temperature ionic liquids (RTILs), are a class of nonmolecular ionic solvents with low melting points. Most common RTILs are composed of unsymmetrically substituted nitrogen-containing cations (e.g., imidazolium, pyrrolidinium, pyridinium) or phosphonium cations with inorganic anions (e.g., Cl\(^{-}\), PF\(_6\)^{-}, BF\(_4\)^{-}). Most of these more common ILs are of limited use analytically. Consequently many ILs containing a variety of cations and anions of different sizes have been synthesized to provide specific characteristics. In this presentation an overview of the structure and properties of ILs and a description of their expanding use in various applications in separations, chromatography and mass spectrometry will be given. A number of studies have appeared indicating that ILs have exceptional promise as stationary phases. They have a dual nature selectivity in that they separate nonpolar molecules as would a nonpolar stationary phase and they separate polar molecules as would a polar stationary phase. Many ILs have exceptional thermal stability. They are being used increasingly in a variety of applications including 2-D GC, enantiomeric separations, the measurement of water in samples/solvents/materials and compact field GC units. ILs have proven to be the best liquid MALDI-MS matrix since we introduced them as such a few years ago. The properties of ILs that make them effective will be discussed. Further, the dications developed for high stability ILs have found another novel use in electrospray ionization (ESI) MS as a reagent for ultra sensitive anion analysis. These will be discussed as well.

**Keywords:** Chromatography, Mass Spectrometry

**Application Code:** Process Analytical Chemistry

**Methodology Code:** Mass Spectrometry
From biology to materials science, advances in microscopy have revealed the chemical and physical structure of surfaces and interfaces as never before. Yet, spatially heterogeneous physicochemical processes at interfaces, underpinning the functioning of living cells, on the one hand, and catalysts, on the other, have proved much more difficult to visualize. We have sought to close the gap between functional and structural microscopy by developing scanning electrochemical probe microscopes that can synchronously reveal activity and topography at the nanoscale and, further, where highly resolved electrochemical data can be combined with information from other microscopy approaches in a correlative multi-microscopy strategy. We make use of nanopipet probes (with dimensions down to a few 10s nm) as mobile electrochemical cells that can be moved quickly and intelligently near to surfaces to gather multiple electrochemical flux signals, to create large data sets that can be presented as images and movies, and can be analyzed and interpreted quantitatively.

We will describe the principles and applications of: (i) scanning electrochemical cell microscopy (SECCM), the smallest and most versatile scanning meniscus technique; and (ii) scanning ion conductance microscopy (SICM), which we have advanced by recasting it as an electrochemical cell, to make a functional microscope of immense power, well beyond its original use. These techniques have been used extensively to reveal the active sites on nanostructured electrode materials, and for patterning and printing in 2D and 3D at the nanoscale. We will further highlight new applications of SICM in cell biology, for visualizing cellular charge and membrane transport processes.

These examples are a roadmap for functional imaging in electrochemistry and interfacial science, where quantitative movies of nanoscale processes in action are predicted to become the norm.
Electroreduction catalysis is a bottleneck for the conversion of low-carbon electricity into chemicals and fuels. New structure–activity relationships are needed to design heterogeneous electrocatalysts with reduced overpotential, higher rates, and enhanced selectivity for CO2 and CO reduction reactions. We are investigating the use of grain boundaries as catalyst design elements for these reactions. Grain boundaries are the interfaces between mis-oriented crystallites in a polycrystalline material. Using bulk electrocatalytic measurements and ex-situ electron microscopy, we have shown that the catalytic activities for CO2 reduction to CO on Au and CO reduction to multi-carbon oxygenates on Cu are directly proportional to grain boundary densities in these materials. Using scanning electrochemical cell microscopy (SECCM), we have revealed that these correlations result from the creation of regions of enhanced catalytic activity at grain boundary surface terminations. The grain boundary catalytic “footprint” is proportional to its strain field, which reflects the concentration of dislocations in the grain boundary vicinity. Our results highlight grain boundary engineering as a promising strategy to access highly active catalysts with persistent surface structures.

Keywords: Electrode Surfaces, Fuels\Energy\Petrochemical
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Microscopy
We report a new kind of microelectrochemical flow system that is well suited for studying electrode modifications, like atomic layer deposition (ALD), that require substrates to have a two-dimensional form factor. The design allows for the electrodes to be modified ex situ and then incorporated directly into the flow cell. The electrodes can be removed after testing and further modified or tested before being reincorporated into the flow cell. Using this flow cell, we have been able to achieve mass-transfer coefficients up to 0.011 cm/s and collection efficiencies up to 57 ± 10 %. We show that it is possible to integrate electrode substrates modified with an ultra-thin layer of ALD Al2O3 and an overlayer of Pt dendrimer-encapsulated nanoparticles (DENs) into the flow cell and evaluate them electrochemically. Additionally, the dendrimer can be removed from the Pt DENs using a UV/O3 treatment, allowing for direct contact with the underlying Al2O3 layer as confirmed by XPS. Subsequently, the product distribution for the oxygen reduction reaction (water vs H2O2) was evaluated in the presence and absence of Pt-Al2O3 support interactions.
Fast scan cyclic voltammetry (FSCV) at carbon fiber microelectrodes (CFMs) has traditionally been used to measure dopamine dynamics in real-time in a variety of physiological systems, most notably \textit{in vivo} in living rodent brains. In the past decade the scope of FSCV has been expanded, by us and others, to \textit{in vivo} measurements of other neuromodulators including serotonin, histamine, adenosine and H\textsubscript{2}O\textsubscript{2}. The goal of these tools is to gather more information about brain function in health and disease. An essential next step towards this goal is to create equivalent tools to measure the most abundant neurotransmitters, glutamate (Glu), acetylcholine (Ach) and GABA, as well as ions such as Ca\textsuperscript{2+} that control synaptic excitation. However Glu, Ach and GABA are not electroactive within the potential window of CFMs and metal ion electrochemistry is ordinarily temporally limited by diffusion. In this work, we take a multi-faceted approach to apply FSCV analysis to these analytes. Specifically, we describe an array of modifications to the CFM surface that challenge the traditional limitations of analysis of these important species. Specifically we will outline chemical modifications using ionophores and molecularly imprinted polymers (MIP). We will describe how covalently bound ionophores enable fast detection of metal ions via a faradaic signal and how MIP modifications facilitate neurotransmitter quantification via changes in capacitative background. A novel design of ionophore will be described that allows neurotransmitter quantification via changes in a redox center upon binding. Finally we present proof of principle that these sensors can be applied \textit{in vivo} in the brain to monitor neurotransmission. In sum, the sheer versatility of FSCV for \textit{in vivo} analysis will be highlighted.

**Keywords:** Bioanalytical, Biosensors, Chemically Modified Electrodes, Neurochemistry

**Application Code:** Neurochemistry

**Methodology Code:** Electrochemistry
Composite carbon electrodes have been known for decades and have important applications ranging from batteries and fuel cells to chemical sensors. In electroanalytical chemistry, screen-printed carbon electrodes have become the most commonly used form of composite carbon. Despite the wide use of composite carbon electrodes, a tradeoff exists between simple fabrication and electrochemical performance, with screen-printed electrodes being easy to make but lacking the performance of high-end carbon materials. Herein, a new method for making composite carbon electrodes using a thermoplastic binder will be presented. The new method allows for straightforward fabrication and patterning using hot embossing, molding, templating, and/or cutting with a CO$_2$ laser. In addition, the electrodes give excellent performance, rivaling that of graphene and high-oriented pyrolytic graphite. In this presentation, a detailed discussion of fabrication methods as well as electrochemical characterization will be provided. The electrodes show significant improvements in electrochemical performance over traditional carbon electrodes (both glassy carbon and screen-printed carbon) with regards to peak current, peak separation, and resistance to charge transfer. The role of metal contamination on the electrochemistry of the carbon electrodes will be discussed. Finally, examples of electrode patterning and integration with microfluidic devices will be provided with an aim toward integrating high performance carbon electrodes into microfluidic sensors.

Keywords: Electrochemistry, Electrodes, Lab-on-a-Chip/Microfluidics
Application Code: Bioanalytical
Methodology Code: Electrochemistry
How does a compound become known as a chemical of environmental concern? In some cases, compounds became known because of a widely-reported environmental contamination incident and because of their known or suspected persistence or toxicity. For example, chlorinated dioxins became well-known because of incidents in Seveso, Italy, and Times Beach, Missouri. In other cases, compounds have gained attention serendipitously. For example, DDT was specifically targeted as a result of Carson’s Silent Spring, polychlorinated biphenyls (PCBs) were discovered during the routine analysis of DDT in fish, and polybrominated biphenyls were discovered during the analysis of PCBs in dairy cow feed. Is this a sustainable approach? Do analytical methods need to focus on previously unsuspected compounds (so-called unknown unknowns)? Do analytical methods need to be developed for specific compounds with high production volumes and chemical stabilities – assuming such knowledge is available? Do analytical methods need to be directed by one or more biological effects? In the past, all of these approaches have played a role. In addition, a new approach called Non-targeted Screening is evolving based on high-resolution mass spectrometry and using several chemical informatics tools. This presentation will give examples of all of these strategies.
Analytical Chemistry and ACS ANYL - New Measurement Approaches for Environmental Sampling and Recent Advances in Analytical Measurements of Emerging Contaminants in Drinking Water

While drinking water is likely safer than it was a few decades ago, due to regulations on disinfection by-products (DBPs) and other chemicals, issues remain. For example, only 11 DBPs are currently regulated in the U.S., but there are nearly 700 currently known, many of which are much more toxic than those regulated. Our source waters are also increasingly impacted by wastewater contaminants, including pharmaceuticals, perfluorinated compounds, pesticides, and other chemicals that are not completely removed in wastewater treatment. Some of these contaminants survive drinking water treatment and are found in our tap water, and others are transformed, some forming more toxic by-products when they react with chlorine or chloramines in treatment. Moreover, new impacts have come on the scene, including impacts of hydraulic fracturing wastewaters that can not only introduce chemicals added during the fracking process, but also high levels of bromide and iodide from natural brines released in these wastewaters. High resolution mass spectrometry with liquid chromatography (LC) and gas chromatography (GC) are helping uncover new DBPs and transformation products in drinking water.

Keywords: Environmental Analysis, Environmental/Water, Mass Spectrometry

Application Code: Environmental
Methodology Code: Mass Spectrometry
Environmental compartments are often contaminated with complex mixtures of pollutants that may display various risks to ecosystems and human health. Currently, high-throughput toxicological screening systems, such as Tox 21 program, are only based on single pure chemical screening, which could not identify key toxic pollutants or evaluate the combined toxic effects from a real complex sample. We therefore developed an integrated toxicology analyzer based on effect-directed analysis, which combined chemical analysis with biological assays for toxicity evaluations on real samples to identify causal toxic chemicals. With the development of novel biological pathway-based biosensors, the toxicity of the extracts of environmental samples could be evaluated. With automated procedure of extraction and fractionation, the complexity of mixture in environmental samples could be rapidly reduced. Thus, the combination of biological assessment and chemical analysis could be applied for the rapid identification of main toxic chemicals in samples. Our system aims to set up a technical platform for effective and rapid evaluation of combined toxic effects as well as the identification of key toxicants in real environmental samples. This system will serve as an important technical support for environmental risk assessment.

Keywords: Environmental Analysis, Mass Spectrometry, Sample Preparation, Toxicology
Application Code: Environmental
Methodology Code: Liquid Chromatography/Mass Spectrometry
Concurrent exposure to a wide variety of xenobiotics and their combined toxic effects can play a pivotal role in health and disease, yet are largely unexplored. Investigating the totality of these exposures, i.e. the exposome, and their specific biological effects constitutes a new paradigm for environmental health but still lacks high-throughput, user-friendly technology. We demonstrate the utility of mass spectrometry-based global exposure metabolomics combined with tailored database queries and cognitive computing for comprehensive exposure assessment and the straightforward elucidation of biological effects. The METLIN Exposome database has been redesigned to help identify environmental toxicants, food contaminants and supplements, drugs, and antibiotics as well as their biotransformation products, through its expansion with over 700,000 chemical structures to now include more than 950,000 unique small molecules. More importantly, we demonstrate how the XCMS/METLIN platform now allows for the readout of the biological effect of a toxicant through metabolomic-derived pathway analysis and further, cognitive computing provides a means of assessing the role of a potential toxicant. The presented workflow addresses many of the methodological challenges current exposome research is facing and will serve to gain a deeper understanding of the impact of environmental exposures and combinatory toxic effects on human health.
Nearly 100 arsenic species, with diverse toxicities, are present in the environment and in biological systems. There is a tremendous analytical challenge in the identification and quantification of trace amounts of individual arsenic species present in complex biological sample matrix. This presentation will highlight recent advances in analytical measurements of arsenic species, including the oxygenated and thiolated, trivalent and pentavalent, and inorganic and organic arsenic species. High performance liquid chromatography (HPLC) separation and simultaneous detection with both inductively coupled plasma mass spectrometry (ICPMS) and electrospray ionization tandem mass spectrometry (ESI-MS/MS) enabled identification and quantitation of individual arsenic species present in various environmental samples. The use of high resolution mass spectrometry further supported the identification of previously “unknown” arsenic species. Development of new molecular probes allowed for novel studies of arsenic binding to proteins. Studies of arsenic speciation and protein binding are critical to understanding the health effects of this top priority environmental contaminant that affects two hundred million people around the world.

References:
Abstract Text

Metal-organic frameworks (MOFs) are presented as a platform material for creating near infrared (NIR) emitting materials. Specifically, a selection of lanthanide ion based MOF materials designed for bioanalytical and biological imaging applications are presented. Various strategies for controlling and systematically tuning the excitation energy of these materials will be discussed. It will be demonstrated that lanthanide-based MOF materials are sufficiently bright for biological imaging applications and that they can be excited at energies that lie within the ideal biological imaging window.

Keywords: Bioanalytical, Material Science, Nanotechnology, Near Infrared
Application Code: Bioanalytical
Methodology Code: Near Infrared
The goal of targeted therapeutics and molecular diagnostics is to accumulate drugs or probes at the site of disease in higher quantities relative to other locations in the body. To achieve this, there is tremendous interest in nanomaterials capable of acting as carriers or reservoirs of therapeutics and diagnostics. Generally, nanoscale particles are favored for this task as they can be large enough to function as carriers of multiple copies of a given small molecule, can display multiple targeting functionalities, and can be small enough to be safely injected into the blood stream. The goal is that particles will either target passively via the enhanced permeability and retention (EPR) effect, actively by incorporation of targeting groups, or by a combination of both. Nanoparticle targeting strategies have largely relied on the use of surface conjugated ligands designed to bind overexpressed cell-membrane receptors associated with a given cell-type. We envisioned a targeting strategy that would lead to an active accumulation of nanoparticles by virtue of a supramolecular assembly event specific to tumor tissue. The most desirable approach to stimuli-induced targeting would be to utilize an endogenous signal, specific to the diseased tissue itself, capable of actively targeting materials. We present the development of nanoparticles capable of assembling in response to selective, endogenous, biomolecular signals. For this purpose, we utilize enzymes as stimuli, because they are uniquely capable of propagating a signal via catalytic amplification. We will describe the preparation of highly functionalized polymer scaffolds, their development as in vivo probes and their utility as a multimodal imaging platform and as drug carriers capable of targeting tissue via a new mechanism. Furthermore, we will describe new methods and approaches for characterizing this kind of dynamic material at the nanoscale, including by liquid cell transmission electron microscopy.
The field of upconversion in ion doped system can be traced back to an idea of Bloembergen in 1959 [1]. Bloembergen proposed that IR photons could be detected and counted through sequential absorption (ESA) within the levels of a given ion in a solid. Role of energy transfer in upconversion was recognized by Auzel in 1966 [2]. Medical science has begun to focus their attention on the use of nanomaterials to improve diagnosis and treatment of diseases with the ultimate goal of moving into personalized medicine. The need to develop more efficient drug delivery procedures motivated us to propose a novel nano-carrier based on lanthanide upconverting nanoparticles (UCNPs). They offer significant advantages in biological applications, particularly the extension of the system applicability to deep tissue regions of the body, a reduced scattering of the excitation wavelength, reduction of auto-fluorescence, and decrease in photodamage to the system under study. We will show relevant biological applications of these upconverting nanoparticles as a platform for drug delivery.

Hybrid Nanostructured Materials for Analytical and Bioanalytical Applications

Nanoscale Metal-Organic Frameworks for Biological Imaging and Cancer Therapy

Metal-organic frameworks (MOFs) are a class of hybrid materials self-assembled from organic bridging ligands and metal ion/cluster connecting points. The combination of a variety of organic linkers, metal ions/clusters, and structural motifs can lead to an infinite array of new materials with interesting properties for many applications. We have taken advantage of this molecular tunability to design nanoscale MOFs (nMOFs) for biological imaging and cancer therapy. We have developed nMOFs as imaging contrast agents. NMOFs possess several interesting attributes, such as high cargo loading capacity, ease of post-modification, tunable size and shape, and intrinsic biodegradability, to make them excellent candidates as imaging contrast agents. I will discuss the use of representative nMOFs in optical imaging (OI), magnetic resonance imaging (MRI), and X-ray computed tomography (CT).

We recently reported the rational design of Hf-porphyrin and Hf-chlorin nMOFs as exceptionally effective photosensitizers for photodynamic therapy (PDT). These nMOFs efficiently generate 1O2 owing to site isolation of photosensitizing ligands and facile 1O2 diffusion through porous nMOFs. Consequently, nMOFs displayed greatly enhanced PDT efficacy both in vitro and in vivo, leading to complete local tumor eradication in the mice receiving a single nMOF dose and a single light exposure. We further combined PDT by a new chlorin-based nMOF, TBC-Hf, and an inhibitor of Indoleamine 2,3-dioxygenase (IDO), encapsulated in the nMOF channels to induce systemic antitumor immunity. The synergistic combination therapy achieved effective local and distant tumor rejection in colorectal cancer models. Although still in their infancy, we believe that the compositional tunability and mild synthetic conditions of nMOF should greatly facilitate their further development for clinical translation.

Primary Author
Wenbin Lin
The University of Chicago

Co-Author(s)

Abstract Text

Biomedical, Nanotechnology

Application Code: Biomedical

Methodology Code: Fluorescence/Luminescence
The luminescence of lanthanide cations possess several complementary advantages over the fluorescence of organic fluorophores and semiconductor nanocrystals, such as sharp emission bands for spectral discrimination from background emission, long luminescence lifetimes for temporal discrimination and strong resistance to photobleaching. In addition, several lanthanides emit near-infrared (NIR) photons that can cross deeply into tissues for non-invasive investigations and that result in improved detection sensitivity due to the absence of native NIR luminescence from tissues and cells (autofluorescence). The main requirement to generate lanthanide emission is to sensitize them with an appropriate chromophore (“antenna effect”). An innovative concept for such sensitization of NIR-emitting lanthanides is proposed herein; the current limitation of low quantum yields experienced by most mononuclear lanthanide complexes is compensated for by using a large number of lanthanide cations and by maximizing the absorption of each discrete molecule, thereby increasing the number of emitted photons per unit of volume and the overall sensitivity of the measurement. To take advantage of this concept, we have created several metal-organic frameworks and succeeded in generating highly emissive NIR MOF reporters. We will discuss their designs, synthesis, structures, photophysical properties and their applications for biological imaging in cells with NIR microscopy.
The Mass Spectrometry community has a long history of developing innovative application to advance a variety of fields. For example, quantitative mass spectrometry of drug substances and metabolite identification has transformed the pharmaceutical discovery and development industry into a more exacting science compared to 40 year ago. Similarly, the integration of mass spectrometry into a variety of “omics” fields over the last 20 years has led to major advancements to our understanding of the dynamics of proteins, metabolites, lipids, etc on cellular function and dysfunction. In all of these cases, the underlying traits that have fueled the advancements are the ever increasing, speed, selectivity and sensitivity of mass spectrometers coupled with the ability to capture and process the data in a robust fashion. More recently, many of these same features of mass spectrometry have emerged as critical elements for integration into the application in high throughput screening. To lay the groundwork for some of the new advancement in this field, a brief history highlighting the use of mass spectrometry for enzyme assays, inhibitor screening and high throughput screening will be presented.

Keywords: Drug Discovery, Enzyme Assays, Mass Spectrometry
Application Code: Drug Discovery
Methodology Code: Mass Spectrometry
MS Based High-Throughput Screening for Drug Discovery

Rapid Metabolite and Xenobiotic Screening Using Automated Solid Phase Extractions and Ion Mobility Spectrometry-Mass Spectrometry

Mass spectrometry (MS)-based technologies are playing a growing role in the analysis of complex samples. Despite significant advances in MS technology, it is currently difficult to obtain measurements with both high throughput and sensitivity for samples with great dynamic ranges such as biofluids and environmental extracts. This problem ultimately results in the inability to effectively account for variation among sample conditions and/or biodiversity, leading to inconsequential findings. To address this challenge, we have coupled an ion mobility separation (IMS) with MS to afford greatly improved measurement throughput, sensitivity, robustness, and quantitative capabilities for rapid analysis of complex samples. Here we showcase the fast small molecule detection method where online Rapidfire solid phase extraction (SPE) is coupled to the IMS-QTOF MS platform. To facilitate the identification of the unknown small molecules in the complex datasets collected from IMS-MS platforms, a database with collision cross sections (CCS) for diverse endogenous and exogenous metabolites was developed. Different classes of molecules, such as sugars, amino acids, lipids, plant metabolites, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) were characterized. This CCS database greatly enables future metabolomic and exposomic studies by allowing others to perform more confident multidimensional identifications and create better tools to thoroughly understand small molecule changes in the biological and environmental systems.

Keywords: Bioanalytical, High Throughput Chemical Analysis, Mass Spectrometry, Metabolomics, Metabonomic
Application Code: High-Throughput Chemical Analysis
Methodology Code: Mass Spectrometry
In 2014, Astrazeneca started a collaboration with Labcyte and Waters to build a prototype high throughput mass spectrometry platform. As a minimum requirement the system should be able to sample at a rate of at least 1 per second which would result in the ability to generate around 85,000 data points per day. This would make the system 8-10 times faster than the current market leading electrospray screening systems available from Agilent.

The heart of this new platform is a modified acoustic dispenser, this generates a droplet spray directly from the well of a standard 384 well plate. The droplet size is comparable to standard electrospray and we believe that the ion is generated via an electrospray type mechanism. This “direct infusion” Echo-MS system can run at sampling speeds of 3 samples per second and enables label-free, high throughput screening (HTS) of biochemical assays.

Our first proof of concept HTS was run mid-2016, the Echo-MS system was able to screen around 300,000 samples. Subsequently we integrated a simple automation solution to feed plates into the system and have now completed a full collection screen of ~2million samples.

In addition to sharing some of the biochemical HTS data from this prototype system we will present recent work demonstrating the potential of this platform into new area of drug discovery.

Keywords: Drug Discovery, Mass Spectrometry, Sample Introduction, Time of Flight MS
Application Code: Pharmaceutical
Methodology Code: Mass Spectrometry
### Abstract Title

**An Open Port Sampling Interface as a Noncontact Injection System for Coupling Liquid Droplet Sample Dispensing Systems with Mass Spectrometry**

### Abstract Text

The open port sampling interface (OPSI) introduce sample into a flowing solvent stream that transports the material into the commercial AP ionization source of the mass spectrometer. In this work, the continuous solvent spillover mode of the OPSI is examined and demonstrated as a non-contact sampling injection system of discrete liquid droplets delivered by a liquid droplet dispensing system. The OPSI uses a vertically aligned, co-axial tube arrangement enabling solvent delivery to the sampling end of the device through the tubing annulus and solvent aspiration down the center tube and into the commercial ESI or APCI emitter probe of a AB Sciex 5600+ mass spectrometer. The ESI and APCI probes were modified to provide higher solvent aspiration rates. A gas displacement or HPLC pump delivered solvent (typically methanol/0.1% formic acid) to the probe. A sampling and dispensing component of the setup was an HTS PAL autosampler (CTC Analytics AG, Zwingen, Switzerland) equipped with an air displacement disposable pipette tip mandrel, a sample plate holder, and a pipette tip rack. Samples comprised of neat vegetable oils, propranolol and propranolol-d7, statin drugs, and drug dosed thin tissue sections were used in this study. The autosampler OPSI combination provides automated sample aspiration and dispensing from microtiter well plates or tissue thin sections onto the OPSI for noncontact injection. The sampling system makes use of commercial disposable pipette tips. Relatively large sample volumes (10-20 μL typically) with drops typically larger than the probe in diameter (several millimeters) are dropped onto the OPSI resulting in an injected volume of 300-500 nL depending on the probe configuration. Sample to sample analysis for liquid sampling is about 40 s. We will discuss the basic operation of the system, injection volumes, sample carryover, matrix effects, and quantitative analysis as well as the ability to do liquid extraction surface analysis.

### Keywords

- Automation
- Mass Spectrometry
- Sample Introduction
- Sampling

### Application Code

High-Throughput Chemical Analysis

### Methodology Code

Mass Spectrometry
Label-free LC/MS based screening technology is routinely used in pharmaceutical industries for hit discovery and various ADME profiling applications. Although the current analysis speed of less than 30 seconds per sample is quite promising, it still cannot match the throughput provided by plate-reader based HTS platforms. In this study direct injection is coupled with an open-port probe (OPP) for direct sampling into a standard ESI ion source. Screening speeds of <2 seconds-per-sample were demonstrated with high sensitivity (attomole loading), good quantitation capability (>3 orders of magnitude), and broad compound coverage (from small molecule pharmaceuticals to peptides and antibodies). The use of a "classic" ESI ion source for MS analysis yielded a perfectly Gaussian-shaped signal peak with baseline width of 0.8 - 1.5 seconds. High sensitivity and reproducibility were demonstrated for this approach, showing linearity over three orders of magnitude, and sensitivity (attomole loading for small molecules, and sub-femtomole loading for intact antibody). The continuous-flow of carrier solvent for the OPP maintained ionization stability and actively cleaned the entire flow system resulting in no observed carry-over. The advantages of this integrated system approach were demonstrated with a Drug-Drug Interaction (DDI) assay, where various substrates/metabolites were monitored and compared to conventional analysis.

Keywords: Drug Discovery, High Throughput Chemical Analysis, Mass Spectrometry, Sample Introduction
Application Code: Drug Discovery
Methodology Code: Mass Spectrometry
Public health laboratories are tasked with analyzing a myriad of sample types for toxic elements. Examples include children’s toys, cultural medicines and personal care products. The elements of most concern are Pb, Cd, As and Hg. Laboratory-based methods such as ICP-MS, ICP-OES, and AAS normally require acid-digestion of the sample. Such methods are laborious, expensive, and require highly skilled staff. Turnaround times are days to weeks. XRF may lack the sensitivity desirable for low-level detection, but recent developments based on doubly-curved crystal (DCC) optics, where low power X-rays from a 5-10 Watts Mo-anode tube are focused into an intense 1-mm spot size, have opened up new possibilities for field studies. Here, we describe a field-based study in which we used an HD Mobile XRF analyzer (X-Ray Optical Systems (XOS), East Greenbush, NY) to analyze personal care products, ethnic foods, herbal medicines and cosmetics among the ethnic Chinese community in the Capital District of NY State. The HD-Mobile utilizes DCC optics to achieve monochromatic micro-XRF thus enhancing measurement intensities. The challenges encountered in field studies include how to “prepare” samples for analysis that are non-ideal for XRF (non-homogenous and irregular surfaces) in a home environment, where time is limited. In this field study, a fast screening technique was deployed: 15 samples prepared and analyzed in ~2.5 hours. The instrument was easily transportable with only moderate effort and was quite straightforward to operate. A QC sample (IAEA-413 Algae) was analyzed in each home yielding between-day reproducibility for the 4 main elements of <10%. Several Chinese medicines were highly contaminated with toxic elements. While the HD-Mobile was found to be useful in the field, it can also play a significant role back in the laboratory, where a quick, non-destructive sample screening process can prevent massive contamination of a much more sensitive ICP-MS instruments.
The interaction of a pulsed laser beam with any sample (laser ablation) creates a transient optical source (plasma), in which chemical information of the sample is contained. Laser-ablation based optical-emission methods [e.g., laser induced breakdown spectroscopy (LIBS) and laser-ablation molecular isotopic spectrometry (LAMIS)] are versatile tools for direct and fast chemical analysis at atmospheric pressure, for virtually any type of sample with minimal sample preparation. Furthermore, the ability to perform standoff or remote analysis is a unique advantage of photon-emission based measurement over other analytical techniques (e.g., mass spectrometry).

It has been recognized long ago that atomic transitions of the same element but from different isotopes emit light at slightly different wavelengths. This isotopic shift allows the different isotopes to be analysed by means of atomic optical spectrometry. Currently, most LIBS measurements are utilized only for elemental analyses; however, LIBS also can provide isotopic information, at least for elements that exhibit large isotopic shifts (e.g., uranium). The development of the LAMIS technique further enhances the capability of laser-induced plasma for isotopic analysis.

LAMIS measures the molecular emission spectra of those radicals that are formed at a late time scale in the plasma, when ablated atoms begin interacting with atmospheric species to create excited-state molecules (e.g., oxides). As molecular spectra typically exhibit two to three orders of magnitude increase in isotopic shift compared to atomic transitions, the shift can be readily measured with a relatively low-resolution optical spectrometer.

In this presentation, the theoretical principles of LIBS and LAMIS for isotopic analysis will be overviewed, the current status (e.g., analytical figures of merit as well as challenges) of the techniques will be examined, and some application examples will be discussed.

Keywords: Atomic Emission Spectroscopy, Atomic Spectroscopy, Laser, Molecular Spectroscopy

Application Code: Other

Methodology Code: Atomic Spectroscopy/Elemental Analysis
New Instrumental Approaches for Remote and Field Applications of Atomic Spectrometry

Pushing the Boundaries of Plasma-Based Chemical Analysis: Alternatives to the ICP that Extend Beyond the Realm of Atomic Spectrometry

The inductively coupled plasma (ICP) has long stood dominant as the most commonly used source for atomic analyses. Despite providing excellent analytical performance, ICP-based methods come with substantial drawbacks: they are expensive, require bulky instrumentation, high radiofrequency power (1–2 kW), and consume large volumes (~15 L/min) of high-purity argon. Accordingly, the search for plasma sources that overcome the drawbacks of the ICP remains an area of active research.

This presentation details two alternative plasmas. First is the microwave-sustained, inductively coupled, atmospheric-pressure plasma (MICAP), a microwave analog to the radiofrequency ICP. In place of the copper load coil of the ICP, the MICAP utilizes a dielectric resonator. When driven with a microwave field, polarization currents are induced in the resonator which generate an oscillating magnetic field that sustains an ICP-like plasma. In contrast to the ICP, The MICAP produces a purely magnetic plasma, free of electrostatic coupling, does not require external cooling, is stable in flowing nitrogen and air, and is powered by an inexpensive microwave-oven magnetron. The second source, the solution-cathode glow discharge (SCGD), is an atmospheric-pressure electrical discharge sustained on a flowing solution in the ambient atmosphere. Unlike the ICP and MICAP, the SCGD is compact, requires no compressed gas, utilizes low, direct-current power (~70 W), and performs analyses by sampling directly from the target solution, thereby obviating the need for a sample-solution nebulizer and greatly reducing memory effects.

Here, recent work towards development and application of the MICAP and SCGD will be presented. Analytical performance and physical features of the MICAP will be compared to those of the ICP. Furthermore, an overview of recent advances towards the application of the SCGD as an ion source for both atomic and molecular spectrometry will be presented.

Keywords: Atomic Emission Spectroscopy, Elemental Mass Spec, Plasma, Plasma Emission (ICP/MIP/DCP/etc.)
Application Code: General Interest
Methodology Code: Atomic Spectroscopy/Elemental Analysis
Portable mass spectrometry (MS) requires stable, sensitive, and low-power ionization sources. However, elemental analysis with MS necessitates high-energy sources to produce gaseous atomic ions from condensed-phase samples. Atmospheric-pressure glow discharges (APGDs) offer exceptional power densities (>500 W/cm³) at lower operating powers (<100 W) that could make them suitable for portable applications. Our group is exploring the use of two APGDs as ionization sources for atomic MS: the flowing atmospheric-pressure afterglow (FAPA) and the solution-cathode glow discharge (SCGD). This presentation will discuss recent progress in using these sources for atomic MS.

The FAPA is a helium-based APGD that is often used for the detection of small, organic molecules. Our group has developed a method to detect elemental ions with FAPA-MS via online complexation reactions with volatile ligands. Thus far, 22 of the 29 tested elements have been detected with detection limits in the femtomole range. But, the open-air nature of FAPA can produce isobaric interferences from ambient species. To overcome this issue, differential mobility spectrometry was used as a post-ionization filter to remove background ions. Ultimately, FAPA-DMS may dramatically improve selectivity and sensitivity in fieldable MS applications.

In contrast, the SCGD is an APGD sustained in ambient air between a metal anode and the surface of a flowing solution. Though SCGD is commonly used as an atomization/excitation source for atomic-emission spectroscopy, we have demonstrated the broad utility of SCGD as an ionization source for atomic, molecular, and biomolecular MS. With regard to atomic MS, SCGD-MS offers detection limits in the sub-ppb range for most elements tested. Further, SCGD was found to be exceptionally stable and, as a result, could be used for precise measurements of elemental isotope ratios. Efforts to create a portable SCGD to couple with portable mass spectrometers will also be presented.

Keywords: Elemental Mass Spec, Instrumentation, Mass Spectrometry, Portable Instruments
Application Code: General Interest
Methodology Code: Mass Spectrometry
**Session Title**: New Instrumental Approaches for Remote and Field Applications of Atomic Spectrometry  
**Abstract Title**: LIBS and LIMS: The Coolest Kids in Town?  
**Primary Author**: José M. Vadillo  
**Author**: Universidad de Málaga  
**Co-Author(s)**: Javier Laserna  
**Date**: Monday, February 26, 2018 - Afternoon  
**Time**: 04:10 PM  
**Room**: 207B  

**Abstract Text**  
The capability of a focused laser beam to induce phase change on a solid surface and generate ions, photons, electrons, neutrals and particles is remarkable. As long as the laser hits the surface above a certain energy threshold, regardless on how distant or close the sample is, we may perform measurements based on the proper collection methods. Optical emission spectroscopy and mass spectrometry are the most common due to their great compatibility with the pulsed scheme of the incoming laser and to the multi-channel nature of the detection when array- or matrix-based optical detectors and time-of-flight analyzers are used.  
The talk will provide a overview on LIBS and LIMS with a focus on problem-solving, emphasizing the amazing versatility when facing complex sampling problems. Thus, examples on remote, on-site and lab configurations will be shown, covering different applied fields.  

**Keywords**: Atomic Emission Spectroscopy, Elemental Analysis, Laser, Mass Spectrometry  
**Application Code**: General Interest  
**Methodology Code**: Atomic Spectroscopy/Elemental Analysis
Current electrophysiology and electrochemistry experiments have provided unprecedented understanding of neuronal activity. However, these techniques are suited for a small, albeit important, panel of neurotransmitters, such as serotonin and dopamine, but these constitute only a subset of the broader range of neurotransmitters involved in brain chemistry. Therefore, the need to develop a technique broadly applicable for the sensing of neurotransmitters near living neurons remains important. Surface-enhanced Raman scattering (SERS) provides a unique opportunity to detect neurotransmitters in close proximity of neurons. We have recently developed dynamic SERS (D-SERS) nanosensors to measure lactate and pyruvate near cells. These nanosensors are based on the decoration of patch-clamp-like nanopipettes with gold nanoparticles, offering a solid support that can be located accurately under a microscope. The nanosensors are thus highly compatible with current physiology experiments also relying on similar nanosensors based on electrochemistry and electrophysiology. In this paper, we will show that D-SERS can measure ATP, acetylcholine, GABA, serotonin, and dopamine, among other neurotransmitters, in a single experiment, with the potential of analyzing a greater number of neurotransmitters. The SERS spectra of these neurotransmitters were identified with a barcoding data processing method and time series of the neurotransmitter levels were constructed. The D-SERS nanosensor was then located near dopaminergic neurons. Detection of ATP and dopamine was realized in a series of K+ depolarization experiments showing elevated levels of both ATP and dopamine. Control experiments were also performed near glial cells, showing only a few basal detection events of ATP and dopamine. This paper demonstrates the potential of D-SERS to detect neurotransmitter excretion events near living neurons.

Keywords: Bioanalytical, Biomedical, Infrared and Raman, Neurochemistry
Application Code: Neurochemistry
Methodology Code: Sensors
Abstract Text
The chemical specificity and high-sensitivity of surface enhanced Raman spectroscopy (SERS) has tremendous promise for biomedical diagnostics. Challenges to utilizing SERS in clinical assays include reproducibility and quantitation. In addition to the challenges associated with SERS, human biofluid samples are complex and contain multiple species that can obscure the signal of the target analyte. To address these challenges, we have demonstrated a sheath-flow interface for SERS detection in flow. This sheath-flow SERS interface uses hydrodynamic focusing to confine analyte molecules eluting/migrating out of a column onto a planar SERS substrate where the molecules are detected by their intrinsic SERS signal. Advantages of the sheath-flow SERS detector include small sample sizes (25 – 100 nL) and reproducible SERS signals. Importantly, the sheath flow environment is resistant to irreversible absorption, which enables high throughput and continuous characterization. We have successfully coupled this detector to HPLC and CE. Work in our laboratory has investigated metabolites and other small molecules present in urine and tumor cell lysates. Results suggest that neurotransmitters can also be detected. Our work suggests a new route to identifying these molecules for diagnostic assays.

Keywords: Bioanalytical, Capillary Electrophoresis, Capillary LC, Surface Enhanced Raman Spectroscopy
Application Code: Bioanalytical
Methodology Code: Vibrational Spectroscopy
Many neurological diseases/conditions are difficult to diagnose in the early stages of disease progression where treatment would be most beneficial. For various conditions, including Parkinson’s disease and mental health disorders, changes in neurotransmitter concentrations could indicate the onset or progression of the disease. Most sampling techniques can detect neurotransmitters in various biofluids, however these techniques often involve multi-step sample preparations coupled with long measurement times. There is a need for the development of sensors for the detection of neurotransmitters that are selective, rapid, and label-free with little to no sample processing. Our group focuses on the detection of biomarkers for neurological activity in non-invasively collected biofluids with enhanced spectroscopies, including surface-enhanced Raman spectroscopy (SERS) and UV resonance Raman spectroscopy (UVRRS). I will present our recent results in neurotransmitter detection.

Keywords: Nanotechnology, Neurochemistry, Surface Enhanced Raman Spectroscopy
Application Code: Neurochemistry
Methodology Code: Vibrational Spectroscopy
In this contribution we report on linear and non-linear Raman spectroscopy to investigate brain tissue focusing on two central contemporary issues of brain tumor research: (I) tumor typing and grading by analysing excised tissue is of utmost importance for detailing a particular therapy plan; (II) for prognostication the tumor has to be removed as completely as possible. While histopathology of excised tissue using haematoxylin-eosin staining is the golden standard for the definitive diagnosis of surgical pathology specimens, this technique is not applicable in vivo.

Here, we will show that linear Raman spectroscopy enables label-free assessment of brain tissues and tumors based on their biochemical composition and allows grading of tumors, determining the primary tumor of brain metastases and delineating tumor margins – even during surgery after coupling with fiber optic probes. While linear Raman spectroscopy shows a high chemical specificity, the size of the region of interest is limited for Raman spectroscopic imaging by the slow acquisition speed. The exposure time can be reduced using non-linear variants of Raman spectroscopy namely coherent anti-Stokes Raman scattering (CARS) and stimulated Raman scattering (SRS) enabling to collect images within video-rate frame rate. Here, we show that CARS- and SRS microscopy are capable of extracting morphochemical information known from HE stained images. These techniques can be applied to investigate native tissue in vivo might open a doorway to an online chemical imaging of brain tissue.

Overall, the presented examples highlight the unique potential of Raman imaging to complement histopathologic assessment of brain tumours.

Acknowledgment
Financial support of the EU, the “Thüringer Ministerium für Wirtschaft, Wissenschaft und Digitale Gesellschaft”, the “Thüringer Aufbaubank”, the BMBF, the DFG, the FCI and the Carl-Zeiss Foundation are greatly acknowledged.

Keywords: Biomedical, Chemometrics, Raman Spectroscopy
Application Code: Biomedical
Methodology Code: Vibrational Spectroscopy
Stimulated Raman scattering (SRS) microscopy is a label-free and noninvasive imaging technique using vibration spectroscopy as the contrast mechanism. SRS has opened a wide range of biomedical applications since it provides instant tissue examination without the need of conventional histological staining, does not affect cell function, and is best suited for imaging small molecules, such as metabolites or neurotransmitters. A major challenge in neuroscience has been to map out the distribution of neurotransmitters in the brain at the subcellular level, which has not yet been possible due to the lack of tools. Recently, we have successfully imaged the neurotransmitter acetylcholine at the neuromuscular junction with SRS microscopy (Fu et al, JACS 2017). However, the sensitivity of current state-of-the-art SRS microscopes is still not high enough to image neurotransmitters in the brain. We have currently set out to improve its sensitivity. The new development aimed at brain imaging will be presented.
Protecting consumers from chemical contaminants in food is pivotal if food industries are to retain consumers’ trust and confidence. This requires a thorough understanding of potential contaminant hazards from raw materials and ingredients, as well as an understanding of the formation of potential toxins that might form during food processing. Modern analytical techniques have improved our awareness of such chemical contaminants, which allows for them to be better controlled.

In this context, the access to analytical tools is pivotal, requiring continuous development and possibly sophistication in terms of identifying appropriate chemical markers (e.g. to understand the source of mineral oil hydrocarbons in food), data acquisition and modelling. Significant progress in portable technologies and their deployment is evident already today, for instance for rapid upstream testing for veterinary drugs and mycotoxins at agricultural level. For food manufacturers, targeted analytical methods complemented by untargeted approaches (e.g. NIR) are part of the control measures at the factory gate when the material is delivered. In essence, testing for food contaminants is an integral part of routine quality control, ideally tailored to the risks in the individual markets / geographies or supply chains. The development of analytical methods is therefore a first step to verify compliance and the authenticity of food materials. A next, more challenging step is the successful establishment of global consensus reference methods.

Therefore, initiatives to drive the alignment of analytical methods at an international level need to be accelerated, that will finally help avoid compliance issues and trade disputes. This requires working closely with all stakeholders and continuously communicating on progress and challenges in a fully transparent manner.

Keywords: Agricultural, Analysis, Biosensors, Food Contaminants
Application Code: Agriculture
Methodology Code: Chemical Methods
### Abstract Text

The domain of analytical instrumentation provides ever increasing capabilities to detect, identify, and quantitate compounds (that may be of interest and sometimes of concern) at lesser and lesser concentrations. An important consideration regarding analytical data collection is the intended use of the data collected. The detection, identification, and quantitation of a compound does not necessarily relate to significance to human food safety, human food nutrition, or the environment. Often mere collection of data generates unnecessary anxiety until potential consequences are understood. In general, the cost of data collection increases while significance decreases as analyte concentrations decrease. Determination of priorities for allocating resources to data collection should include the relevant value of the information gathered.

### Keywords:
- Detection, GC-MS, Identification, Liquid Chromatography/Mass Spectroscopy

### Application Code:
- Food Safety

### Methodology Code:
- Gas Chromatography/Mass Spectrometry
Reducing Threats to Food Safety, Farm to Fork: Advances in Instrumental Applications

Identification of Strain Specific Bacterial Proteins by Top-Down and Bottom-Up Mass Spectrometry

Typing and trace-back of bacteria and bacterial toxin contamination in the food supply requires a high level of specificity. While MALDI-TOF MS has emerged as a rapid method for routine identification of microorganisms by mass spectrometry, commercial methods are generally not applicable to the subspecies and serovar level identification needed to differentiate Salmonella. The combination of ESI-LC-MS generated intact protein expression profiles and top-down mass spectrometry provides a robust platform for identification of protein markers that result from serovar specific non-synonymous SNPs, without the need for a sequenced genome. Combinations of marker proteins can also be used in assays for rapid and sensitive differentiation of bacteria at the peptide level by bottom-up mass spectrometry in a food matrix. These methods are also applicable for differentiation of closely related bacterial protein toxins.

Keywords: Food Safety, Mass Spectrometry, Protein, Proteomics

Application Code: Food Safety
Methodology Code: Mass Spectrometry
Both nonylphenol isomers (NPs) and Bisphenol A (BPA) are persistent environmental contaminants and the prominent examples of the endocrine disrupting chemicals (EDCs) that adversely impact the human reproduction and neurobehavioral development. The contamination of food products by NPs and BPA raise public and regulatory concerns. Herein, we established a simple, reliable and cost-effective method to quantify both BPA and NPs in various food and beverage matrices. The method combined the salting-out assisted liquid/liquid extraction with acidified acetonitrile and the freeze-out removal of the co-extracted lipids before the analysis by LC-MS/MS in negative ESI mode. This method has been validated on various food matrices, including commercially packaged beverages, infant formula products and ingredients with acceptable accuracy and precision. The limit of quantification (LOQ) is 0.3 ng/g (BPA) and 10 ng/g (NPs). Satisfactory recoveries were obtained for both BPA and NPs in the range of 92 – 127% in the validated food matrices. The triplicate sample spiked at LOQ level over three days in each food matrix yielded the interday precision in the range of 1.9 – 11.5%. Moreover, the performance of this method meets the proposed AOAC standard method performance requirements (SMPRs) for “Determination of Bisphenol A (BPA) in Commercially Packaged Ready to Consume Carbonated and Non-carbonated Water and Non-Alcoholic Beverages”.

Keywords: Food Contaminants, Food Safety, Liquid Chromatography/Mass Spectroscopy

Application Code: Food Safety

Methodology Code: Liquid Chromatography/Mass Spectrometry
Historically, pesticide residue screening has been confined to limited and targeted lists. This was due mostly to instrument capabilities. Today, high resolution mass spectrometry and other techniques has opened up the door to a capacity for a laboratory to rapidly expand the number of analytes screened. Not uncommon anymore are screens of 200+ compounds. In our lab, the official count is about 500. We will show how we developed this screen, explain the lessons learned, the impact it had on our regulatory program and on food safety.
It is well established that individual cells, even from the same origin, differ from each other in many aspects due to stochastic biological processes and differences in environmental perturbations. Cell heterogeneity has been found to play an important role in many biological processes, including cellular differentiation and immune response, as well as disease development. The heterogeneity of cells in culture and in organisms poses a challenge for many experimental measurements. Traditional ensemble analysis based on averaging a large population of cells, as a result, masks the behavior of minority subpopulations and effectively blinding researchers to possibly interesting differences between cells. Single-cell analysis is an important and emerging field that gives insights into heterogeneity between cells and advanced cellular processes at high resolution, which is important for cancer research, regenerative medicine, immune system research and diagnostics, as well as for the production of therapeutics. Microfluidics has proven to be a leading tool for single cell analysis since device dimensions are on the same scale as those of cells, allowing for precise fluid and cell manipulation at high throughput. In this talk, I will present our recent efforts on developing microfluidic technologies for high throughput single cell isolation, manipulation, and analysis at the DNA, RNA and protein level with single-molecule sensitivity.
Although conventional bulk analysis is commonly used, it often overlooks rich information available only when single cells are studied. Due to cellular heterogeneity, averaging end-point results from a population of cells may result in misleading results. Therefore, real-time single-cell analysis plays an essential role in revealing dynamic cellular heterogeneity. We have developed multiple microfluidic platforms to study dynamic intracellular calcium mobilization pertaining to drug efficacy and drug safety at the single-cell level. Along with experimental studies, we studied flow behaviors on single-cell manipulation and shear stress reduction in microfluidic chips using computational fluid dynamics (CFD) simulations. The simulation results have not only explained flow phenomena observed in experiments but also predicted new flow phenomena, providing guidelines for new chip design and optimization and a better understanding of the cell micro-environment and fundamentals of microfluidic flows in single-cell manipulation and analysis. In addition, we proposed a new concept—Same-Single-cell Analysis (SASCA)—in the field of single-cell analysis to address cellular heterogeneity issues in dynamic multidrug resistance studies by using the same cell as its control. Since only a limited number of cells are required in SASCA, SASCA has enormous potential to look for personalized drugs as a prognostic method. Lastly, efforts have been made to exploit the potential of single-cell genetic analysis for forensic applications.

Financial support from NIH, NSERC, NIJ, NSF-PREM, UT STARS Award, and IDR and URI Awards from UTEP is gratefully acknowledged.

Keywords: Bioanalytical, Biosensors, Biotechnology, Lab-on-a-Chip/Microfluidics
Application Code: Bioanalytical
Methodology Code: Microfluidics/Lab-on-a-Chip
Despite recent advances in single-cell genomic, transcriptional and mass cytometric profiling, it remains a challenge to collect highly multiplexed measurements of proteins produced from single cells for comprehensive analysis of immune functional state. I will discuss novel technologies for single-cell proteomic profiling, in particular, a microchip technology for co-detection of 40+ immune effector proteins such as cytokines/chemokines at the level of single cells, representing the highest multiplexing recorded to date for a single-cell protein secretion assay. I will present how this technology was conceived at the beginning, evolved over generations, and eventually being in the pipeline of commercialization by IsoPlexis, which can help pharmaceutical companies like Novartis, Kite Pharma, and Juno Therapeutics to evaluate their immunotherapy products. This microchip technology allowed for the full-spectrum dissection of T cell functions including genetically engineered chimeric antigen receptor T cells (CAR-T) in the treatment of patients with acute lymphoblastic leukemia or non-Hodgkin’s lymphoma. Our data obtained from a medium-scale cohort in the clinical trial of CD19 CAR-T cells demonstrated strong association between CAR-T cells’ polyfunctionality (the ability for a single T cell to co-produce multiple immune effector proteins) and patient responses, which opened up the opportunity for predicting not only therapeutic efficacy but also potentially life-threatening immunotoxicity. All these underscore the importance of measuring functional proteomic heterogeneity even in phenotypically identical cell populations in order to evaluate the quality of cell-based therapeutics or to monitor patient responses for precision medicine.

Keywords: Bioanalytical, Biomedical, Biosensors, Clinical/Toxicology
Application Code: Biomedical
Methodology Code: Microfluidics/Lab-on-a-Chip
This talk will describe the use of microfluidic technology to control and manipulate drops whose volume is about one picoliter. These can serve as reaction vessels for biological assays. These drops can be manipulated with very high precision using an inert carrier oil to control the fluidics, ensuring the samples never contact the walls of the fluidic channels. Small quantities of other reagents can be injected with a high degree of control. The drops can also encapsulate cells, enabling cell-based assays to be carried out. The use of these devices for biotechnology and diagnostic applications will be described.

Keywords: Biotechnology, Genomics
Application Code: Genomics, Proteomics and Other 'Omics
Methodology Code: Microfluidics/Lab-on-a-Chip
Biosystems are intrinsically heterogeneous. Conventional ensemble measurements heavily rely on ‘average’ values from multiple experimental replicates to study the quantitative difference between samples, or between conditions. However, the heterogeneity at the cellular level make such strategy impossible in many cases. Next generation sequencing technologies allow us to obtain large and informative data-set with relatively low cost and high throughput, and become the key driving force to facilitate our understanding of complex biosystems. However, prevalent methods are still problematic when handle highly heterogeneous samples because of the mix of real biological variations and technical variations or noises. I am going to present recent technology developments in my group, mostly the combination usage of microscopic imaging with microfluidics, to facilitate the approaches using next generation sequencing tools to analyze small number of cells or even single cells.
The Twenty-Ninth James L. Waters Symposium - Ambient Ionization Mass Spectrometry for Imaging a
DESI: Concept, Early Developments, Current Trends

This presentation traces the evolution of ambient ionization methods (ionization in the ambient environment without
sample preparation). Special emphasis goes to DESI and paper spray ionization. The talk covers the basics of these
methods and their applications to pharmaceutical and clinical analysis. Special emphasis goes to quantitative
measurements. Applications of DESI in imaging also get special mention as do derivatization reactions which can
accompany DESI and which led to the study of accelerated chemical reactions in microdroplets. Recent trends are
represented by (i) synthesis at the 10's mg scale of organic compounds using accelerated droplet reactions and (ii) the
ability to screen microtiter plates at rates of 1 reaction well per second. The implications of this last capability in
automated synthesis are noted.

Keywords: Imaging, Instrumentation
Application Code: Bioanalytical
Methodology Code: Mass Spectrometry
**Abstract Title**
From Discovery to Commercialization: The Journey of Desorption Electrospray Ionization (DESI) Mass Spectrometry

**Primary Author**
Justin M. Wiseman
Prosolia, Inc.

**Abstract Text**
Guided by the vision of ambient mass spectrometry that simplifies workflows for molecular analysis, a start-up company – Prosolia, turned a simple graduate student experiment into a commercial enterprise. From the invention of DESI to the present commercial successes and challenges and future opportunities will be described.

**Keywords:** Imaging, Lipids, Mass Spectrometry, Metabolomics

**Application Code:** General Interest

**Methodology Code:** Mass Spectrometry
While significant advances have been made in proof-of-principle approaches in atmospheric ionization, they are not yet used by the general population. Yet, and although still far away, the future potential exists that one day every person with a smart toilet, smart mirror and if size of instrumentation can be solved, a smart phone, will perform molecular analysis of any object/sample they want. This information will then be collected and we gain global insight into the molecular diversity that exists in the world and their distributions. When such a routine analysis becomes a reality, we will change what we eat, how we preserve food, how we prepare clothing and construction materials, how we exercise, and how we approach health. But what would a potential roadmap look like to achieve such amazing goal? How do we make the data that is collected more informative? How do we reuse such information to enhance our molecular understanding? How did Google, Amazon or Facebook achieve this for text searches? While it is clear that such capabilities do not yet exist for atmospheric methods, we will highlight the potential with atmospheric ionization experiments from our own laboratories (DESI, NanoDESI, Flow probe and other integrative atmospheric techniques). We will further demonstrate the approach to data reuse, including showcasing that data from non-atmospheric ionization methods and new data analysis strategies, can be used to leverage discoveries with atmospheric ionization. This will be a key requirement to make the information from atmospheric ionization techniques usable for the larger community on a daily basis. This will eventually become as common as a Google search done today but instead of a text search, it will be a simple mass spectrometric scan of a sample without any sample preparation.

**Abstract Text**

While significant advances have been made in proof-of-principle approaches in atmospheric ionization, they are not yet used by the general population. Yet, and although still far away, the future potential exists that one day every person with a smart toilet, smart mirror and if size of instrumentation can be solved, a smart phone, will perform molecular analysis of any object/sample they want. This information will then be collected and we gain global insight into the molecular diversity that exists in the world and their distributions. When such a routine analysis becomes a reality, we will change what we eat, how we preserve food, how we prepare clothing and construction materials, how we exercise, and how we approach health. But what would a potential roadmap look like to achieve such amazing goal? How do we make the data that is collected more informative? How do we reuse such information to enhance our molecular understanding? How did Google, Amazon or Facebook achieve this for text searches? While it is clear that such capabilities do not yet exist for atmospheric methods, we will highlight the potential with atmospheric ionization experiments from our own laboratories (DESI, NanoDESI, Flow probe and other integrative atmospheric techniques). We will further demonstrate the approach to data reuse, including showcasing that data from non-atmospheric ionization methods and new data analysis strategies, can be used to leverage discoveries with atmospheric ionization. This will be a key requirement to make the information from atmospheric ionization techniques usable for the larger community on a daily basis. This will eventually become as common as a Google search done today but instead of a text search, it will be a simple mass spectrometric scan of a sample without any sample preparation.

**Keywords:** Informatics, Mass Spectrometry

**Application Code:** Laboratory Management

**Methodology Code:** Mass Spectrometry
Development of ambient ionization techniques in the past decade has led to the establishment of a new discipline, namely, synthetic mass spectrometry. This method of synthesis has proven to be more efficient than conventional bulk methods, for a large number of organic reactions. Several studies have demonstrated the creation of compositionally precise nanostructured materials using ions. Synthesis, catalytic activity and large area patterning of bare, uniform noble metal nanoparticles were demonstrated using ambient electrolytic spray. The same methodology can be extended to electrospray deposition of metal ions on surfaces to create uniform nanobrushes composed of oriented one-dimensional silver nanowires (NW) with aspect ratios of 102 – 104. These structures can be grown over large (cm2) areas over conducting surfaces. The materials synthesized can have functional attributes such as catalysis and superhydrophobicity. Such structures may be used to create intense electric fields resulting in the emission of molecular ions. Such ionization occurring even at 1V can help in the minaturization of mass spectrometers. Using such approaches, fragile species such as transition metal complexes can be detected. In the very recent past such ion based methods have been used to make 2D metals. Synthesis methods have been extended to make devices capable of atmospheric water capture.

**Keywords:** Mass Spectrometry, Nanotechnology

**Application Code:** Nanotechnology

**Methodology Code:** Mass Spectrometry
Surgical resection (removal) is the main curative option for many solid cancers. The extent of cancer resection is commonly assessed during surgery by histopathologic staining and evaluation of (frozen sections of) the tissue at the specimen margins to verify whether cancer is present. In this lecture I will compare this method to an alternative procedure being developed in my laboratory and several others, called desorption electrospray ionization mass spectrometric imaging (DESI-MSI). A thin slice of tissue is mounted on an XY translation stage and bombarded with microdroplets that can dissolve lipids and other small-molecule metabolites. The resulting splash of droplets enters a high-resolution mass spectrometer allowing generation of a chemical map, which can be analyzed to distinguish cancer from benign tissue. Results are compared to standard histopathologic determinations and appear to offer the promise of superior performance, both in accuracy and in time.

**Keywords:** Electrospray, Mass Spectrometry

**Application Code:** Biomedical

**Methodology Code:** Mass Spectrometry
Data Science in the Chemistry Curriculum

Some Examples of Applied Statistics to Problems and Experiments from the Undergraduate Analytical Chemistry Curriculum

This presentation will focus on application of statistical methods and techniques to selected problems and experiments from the author’s quantitative and instrumental analysis lecture and laboratory course sequences, and the upper-level elective course in chemometrics, at the University of Pittsburgh – Greensburg. The focus of instruction in these courses has progressed more and more toward analytical data treatment, particularly the use of statistics for analysis of quantitative data and results from various problems and experiments performed in these courses. Such emphasis on analytical data treatment is driven by the need for undergraduate chemists to become more familiar and comfortable with the use of statistical methods and techniques for objective treatment of analytical data and results, and to become prepared for working with such methods and techniques for data analysis in the professional workplace. Examples of application of statistics to data analysis scenarios from the author’s quantitative/instrumental analysis and chemometrics courses will include (but are not limited to) the use of ANOVA for testing of significance of results from determination of alkalinity in mine drainage or selected metals in tea types, assessment of univariate calibration data from determination of various analytes in different samples, and an attempt at multivariate calibration/validation for simultaneous determination of iron and aluminum (an ongoing project in the author’s group). The use of specific software packages, e.g. Microsoft® Excel™ and MATLAB®, for analytical data treatment will also be discussed.

Keywords: Chemometrics, Software, Statistical Data Analysis, Teaching/Education

Application Code: Other

Methodology Code: Chemometrics
The separation sciences course at Seattle Pacific University introduces students to applications of chemometric techniques for the analysis of complex samples, specifically principal component analysis (PCA) and partial least squares analysis (PLS). For three years that the course was offered, students performed a multi-day gas chromatography mass spectrometry (GC-MS) experiment to model and make predictions about fuel samples using PLS in MATLAB and we published a Journal of Chemical Education (JCE) article describing this. The course curriculum was later simplified to include only a lecture, a literature assignment, and a simplified version of the JCE experiment. The simplified version of the experiment still allows students to run the GC-MS instrument and obtain chromatographic fingerprints of the complex samples but the simplifications include removal of some of the challenges of handling data outside of the instrument software, reduction of the number of samples to be run, and therefore fewer chromatograms to be analyzed. The students learn that chemometric applications require good decisions and clear assumptions to maintain data integrity while attempting to separate irrelevant noise from important chemical signals. Those decisions and assumptions often involve applying appropriate preprocessing steps such as normalization and background correction techniques without adding artifacts and while trying to maintain objectivity. In a different course, the analytical spectroscopy course, students are introduced to classical least squares resolution of overlapping peaks in the spectra of complex samples. Additionally, individual students perform independent research projects involving chemometric analysis of various types of complex samples. Ultimately, the goal is for students to learn about some of the main applications and limitations of chemometrics applied to chromatographic and spectroscopic data for the analysis of complex samples.

**Keywords:** Chemometrics, Fuels\Energy\Petrochemical, GC-MS, Teaching/Education

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

**Methodology Code:** Gas Chromatography/Mass Spectrometry
A common experiment in the many undergraduate courses in analytical chemistry is the quantitative analysis of a two-component mixture by UV/Visible spectrophotometry. The analysis typically involves measuring a sample’s absorbance at two wavelengths, determining the molar absorptivity for each analyte and each wavelength, and using Beer’s law to solve for the concentration of the two analytes simultaneously. Rarely is this analysis extended to more than two components or to data collected at more wavelengths than there are analytes. In this presentation, we will consider how to use the analysis of multicomponent mixtures to introduce undergraduate students to principal component analysis, cluster analysis, and multiple linear regression using the statistical programming language R.
Introducing Undergraduates to Data Science through Interdisciplinary Research

While there is no accepted definition of data science, it is generally considered an interdisciplinary field involving statistics, algorithms/models, visualizations, and domain knowledge “in order to solve analytically complex problems.” In chemistry applications, data science or chemometrics, is becoming more relevant as faster instruments with multidimensional outputs continue to provide more and more data. With data science becoming more relevant both inside and outside of STEM fields, we at Westminster College saw a need and opportunity to equip our students with some knowledge and skills in data science that might be useful as they pursue graduate school or careers. Our foray into data science began as an interdisciplinary collaboration among chemistry and math students and faculty members. Through mentoring undergraduate research, we faculty members learned data science side-by-side with our students. The students have pursued research projects involving big data sets of spectroscopic data, weather data, trace metal concentrations, and survey data. They have used the commercial software package Solo (Eigenvector Research, Inc.) and the open source RStudio to process their data by means of basic statistical methods, principal component analysis, and multivariate regression. This presentation will describe the interdisciplinary research process and the results of some of the undergraduate projects, as well as some resources and tools that were helpful for doing data science. We will also discuss how this collaborative work has evolved from interdisciplinary undergraduate research to a single course in data science and now to a proposal for a data science minor at Westminster College.

Keywords: Atomic Spectroscopy, Chemometrics, Education, Statistical Data Analysis

Application Code: Other

Methodology Code: Education/Teaching
Pre-filled syringes (PFS) are increasingly becoming a container of choice for storing and administering pharmaceutical products. PFS components and residues from processing tools may leach organic and inorganic chemicals into formulated drugs, as extractable and leachable compounds. As part of safety risk assessment, it is very important to identify and quantify those extractables and leachables as they may pose safety risks to patients and/or change the efficacy of the medical products.

This presentation will focus on a case study regarding the extractable and leachable testing of PFS for a drug formulation containing high content of castor oil. The choice of the extraction solvent systems and study design to bracket and mimic hydrophobicity and administration of drug formulation will be discussed. In order to obtain a comprehensive extractable profile, multiple analytical techniques were used to identify and quantify the extractables, including Headspace (HS)-GC-MS/FID analysis for volatile organic compounds, GC-MS/FID analysis for semi-volatile organic compounds, LC-MS/UV analysis for non-volatile organic compounds, and ICP-OES analysis for trace elements. This presentation will show that internal database and High Resolution Accurate Mass (HRAM) data facilitate confident compound identification and unknown compound structure elucidation. Analytical challenges associated with the drug formulation containing high amount of castor oil during the leachable testing will also be discussed.

**Keywords:** Biopharmaceutical, GC-MS, Liquid Chromatography/Mass Spectroscopy, Pharmaceutical

**Application Code:** Pharmaceutical

**Methodology Code:** Mass Spectrometry
Extractables and Leachables Analysis

Practical Approaches of Extractables/Leachables Study and Toxicological Assessment on Label Materials for Drug Products

Plastic Container Closure Systems (CCS) including the label materials are the primary choice of packaging system for the parenteral drug products. However, there are challenges of designing extractables/leachables (E/L) studies and safety assessment for container closure materials as well as label materials due to the large volume dose of the parenteral drug products and the low level of leachables at ppb (ng/mL) to ppm (µg/mL).

The Label Materials (such as adhesive, paper, ink, varnish, etc) are defined as “no direct contact” materials Per USP <1661>, which do not come into direct physical contact with drug products. However, depending on the container permeability and the label application process on the containers, the label materials can be considered as “potentially interacting” components. The requirements for E/L studies and safety/toxicological evaluation per 21 CFR 210 and 211 are applying to label materials. The safety assessment should be specifically discussed in Modules for Toxicology Written Summary/Other Toxicity of the ANDA/NDA submission.

The approach for extractables/leachables studies as well as the toxicological evaluation of the safety of leachables must be based on good scientific principles and take into account the specific container closure system, drug product formulation, dosage form, route of administration, and dose regimen.

This presentation will review the basics of printing techniques, composition and chemistry of label materials, possible interactions of label materials with the packaging materials and the drug formulations. The presentation will discuss how to design label material E/L studies with analytical techniques such as HPLC/PDA/MS, GC/FID/MS, etc., the evaluation of E/L study results, and how to conduct safety assessment particularly for label materials used for the parenteral drug products through case studies.

Abstract Text

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Plastic additives are used during manufacturing to establish appropriate mechanical and chemical properties. However, these additives can often leach into the final consumer product. The analysis of extractable and leachable compounds from plastic is required in numerous fields, including both the pharmaceutical and food safety industry. In the materials characterization workflow, routine testing of the packaging is an important step to demonstrate batch to batch reproducibility of the extractables profile. Therefore a robust analytical method for testing is needed.

The principles and benefits of analytical method development for an extracted low density polyethylene sample are shown and a final LCMS method using a UPLC system and a tandem quadrupole mass spectrometer was developed. The method was then transferred to a UHPLC system with a single quadrupole mass detector, consistent with the needs of a QA/QC environment.

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

**Application Code:** Polymers and Plastics

**Methodology Code:** Liquid Chromatography/Mass Spectrometry
Lipid emulsions are widely administered as a source of essential fatty acids in parenteral nutrition. They also have a high potential to extract compounds from plastic packaging. Unfortunately, the complexity of such formulations impedes the monitoring of container leachables in the drug product. Here, a fast and sensitive LC/MS/MS method has been developed for simultaneous quantitation of representative leachables in lipid emulsion matrices. Formulations containing up to 20% lipid were treated with 2,2-dimethoxypropane (DMP) to de-emulsify the emulsion prior to sample analysis. Upon reaction with DMP, the aqueous portion of the emulsion was converted to acetone and methanol, with almost all organic leachables residing in the organic layer. Polar components remained with any residual water in a reduced-volume aqueous layer. Leachables in the organic layer were analyzed using a targeted LC/MS/MS method designed to mitigate matrix effects. The optimized LC/MS/MS method combined core-shell column technology and a sophisticated strong organic solvent wash to produce satisfactory results for all five target leachables. The method was validated for several lipid-containing matrices. The performance characteristics of the developed method were established by assessing accuracy, response linearity, precision, specificity, and solution stability. The method was determined to be suitable for the quantitation of 2-EHA and p-TSA over the range 0.8 - 8 mg/L, 25-crown-5 from 0.5 - 5 mg/L, Irganox 1010 from 0.25-2.5 mg/L, and BHT from 4 – 40 mg/L. The total LC/MS run time was 23 minutes and sample preparation was straightforward as no centrifugation or evaporation/reconstitution steps were involved in the DMP reaction.
Lack of transparency within any food system takes a tremendous toll on individuals no matter where you live. Rich or poor, it affects everyone. Every day people are unwittingly being cheated financially and nutritionally as a result of food systems that we have become emotionally detached from and yet physically unable to escape.

**Categories of Food Transparency issues:** The combination of these issues amplifies the problem:

- Food labeled as something that it isn’t
- Misrepresented freshness and nutritional quality
- Significant nutritional value is lost in the supply chain
- Significant nutritional value is further lost in the food preparation process

What we have learned over the past several years is that, contrary to popular beliefs, retailers are desperate to solve these issues on behalf of their customers. We’ve also learned that, while it is possible to significantly solve food fraud incidents by using various field spectroscopic instrumentation solutions, the answer doesn’t lie within a “device.” The combination of analytical chemistry, high-fidelity lab-level spectroscopy, and modern machine learning data techniques to allow a single instrument platform to work quickly across many different types of single-ingredient foods is possible. We believe that providing a workable spectroscopy-based solution to the world’s largest retailer supply chains is the right place to effect the biggest positive change. Massive industry players are best positioned to exert pressure back through the food chain. But this happens only if we deliver a sensible, workable solution specifically design for their existing processes.

This paper will discuss how we are approaching this novel development solution while utilizing known, proven techniques and instruments.

**Keywords:** Food Identification, Spectroscopy

**Application Code:** Food Identification

**Methodology Code:** UV/VIS
Hand-held Laser Induced Breakdown Spectroscopy (HH-LIBS) was first introduced in a true hand-held form for scrap metal sorting in 2013. With both advantages and disadvantages versus the incumbent technology in that space, hand-held X-ray Fluorescence Analyzer, HH-LIBS continued to carve out a small, but important, niche of that space due to its light element capability, low cost of ownership and no licensing requirements. Today, due to aggressive investment into miniature laser and spectrometer technology, HH-LIBS has expanded its capability beyond traditional scrap sorting application. Additional applications, once only possible with laboratory analyzers, is now a reality in the field portable device. This paper will introduce several new applications of interest with hand-held LIBS including beryllium decontamination, forensic analysis of metal fragments, carbon in steel analysis and lithium in aluminum aerospace alloys.
Security and forensic applications such as explosives, hazmat and narcotics screening are being transformed by high-speed spectral imaging. Traditional screening methods involving laboratory benchtop analysis are time consuming and require sample collection and transportation, with the ever-present possibility of breaking the evidence chain. Fiber-optic based spectroscopic instruments can perform on-the-spot analysis. However, these point source spectroscopy systems cannot image directly, and image mapping via point rastering can be very slow. Additionally, to obtain high spatial and spectral resolution, these instruments must use small fibers and narrow slits, greatly decreasing system throughput and increasing the acquisition time required to obtain high quality data.

Free-space hyperspectral imagers, by contrast, can directly image security and forensic samples with high speed, high sensitivity and high throughput. Spatial resolution is limited by the sensor pixel pitch, rather than fiber diameter, and image mapping by pushbroom sampling can typically be done in less than the square root of the time required by point rastering. High spectral resolution and high signal-to-noise can be obtained simultaneously using the High Throughput Virtual Slit, an innovative optical design. Raman line area imaging takes full advantage of modern high power diode laser technology. By shaping the excitation beam into a line, rather than a point, sample photobleaching and unwanted photochemistry are eliminated and mapping speed is greatly enhanced. With hardware limitations to data acquisition removed, data transfer and file size become analysis bottlenecks. We will present spectral image data and discuss thresholding and other data reduction strategies that can improve the speed, ease of use, sensitivity and specificity of security and forensic image analysis.

**Keywords:** Identification, Imaging, Instrumentation, Raman Spectroscopy

**Application Code:** Homeland Security/Forensics

**Methodology Code:** Portable Instruments
As portable and handheld Raman spectrometers are gaining acceptance in the field, users are beginning to demand higher and higher levels of performance. This is in large part due to the drive to expand the application space to include applications such as medical diagnostics, gemology/minerology, and even laboratory research in addition to traditional applications such as incoming inspection and unknown material identification. In this presentation, we will discuss a novel method that shows a great deal of promise for increasing the spectral range, resolution, sensitivity, and background removal in portable and handheld Raman systems through the use of two different laser wavelengths in the same package. For this reason, we developed a new compact laser package containing two electro- and thermally isolated laser diodes which wavelength stabilized, collimated, and co-linearly beam combined. This new laser package enables systems designers to utilize a wide variety of techniques that are not possible with a single wavelength. Several examples will be covered including spectral concatenation, background decomposition, and crystallography.

**Keywords:**  Laser, Raman Spectroscopy

**Application Code:**  Biomedical

**Methodology Code:**  Portable Instruments
Handheld mass spectrometry continues to mature and expand its reach in focused safety & security applications, and recent work has expanded its role in life-science assays in conjunction with micro-scale liquid-phase separations. We’ll review the current state of the art in this technology (both the MS and separations) with illustrative application data, along with opportunities for more advanced roles in POC.

Keywords: Capillary Electrophoresis, Chemometrics, Mass Spectrometry
Application Code: Safety
Methodology Code: Mass Spectrometry
Miniature NIR Spectrometers Extend the Reach of NIR Spectroscopy

Tiny and economical MEMs sensors in the NIR are playing a key part in the Internet-of-Things (IoT) evolution. Measuring only millimeters in dimension, the Fabry-Perot (FP) sensors first developed at VTT in Finland have undergone many generations of improvements, becoming more stable, faster, and more rugged with each generation. Here we discuss applications of the Spectral Engines FP sensor in consumer devices and in process analytical control. In consumer devices, we give examples such as fabric analysis and food identification, including computation of energy content and basic macronutrients. Many of these measurements have been implemented with a cloud-based calibration solution. In process measurements, applications such as moisture, blending, and polymers have all been popular. In this presentation we will focus on performance figures of the current generation of NIRONE sensors, implementation of the sensors, and recent results.

Abstract Text

keywords: Chemometrics, Instrumentation, Near Infrared
Application Code: General Interest
Methodology Code: Near Infrared
A method is developed that expands the applicability of Raman to See_Through diffusely scattering media such as opaque packaging materials, as well as to thermolabile, photolabile, or heterogeneous samples.

Traditional Raman fiber optics probes employ a focused design similar to confocal microscopes. The excitation light emerging from the laser fiber is collimated and then focused on the sample by a focusing optics, and the same optics also collimates the Raman signal beam scattered by the sample with another lens focusing it to a signal collection fiber. The sample is usually placed at the focal plane of the focusing lens. In this way excitation power density and consequently the Raman signal density is maximized at the sampled volume, and only signal from this tightly focused volume is collected by the collection fiber. This confocal design has the advantage of maximum throughput, and can be used to measure samples inside transparent containers in the same way a confocal microscope does optical sectioning. However, when the container diffuses the light strongly, light can no longer be focused inside the container, the confocal approach loses its effectiveness.

The See_Through Raman Analyzer developed by B&W Tek illuminates and collects the Raman scattered light from a large sample area. This greatly increases the effective sampling depth, allowing the measurement of material inside visually opaque containers. The large area also has the additional advantage of preventing sample damage by reducing the power density, as well as improving accuracy by eliminating heterogeneous effect. With its high throughput design, the See_Through analyzer provides identity of common chemicals through thick packaging material in seconds. For normal measurements, a range of accessories can be used to take advantage of the high throughput design for different applications including microscopy and stand-off Raman.

Keywords: Identification, Raman Spectroscopy
Application Code: Homeland Security/Forensics
Methodology Code: Molecular Spectroscopy
Here we demonstrate a handheld spectrometer using a smartphone for illumination, imaging, and data processing capable of replicating 3 major biosensing modalities in a portable, self-contained system. A custom fiber optic assembly allows for the switching between the smartphone flash and an on-board laser diode for illumination of colorimetric absorption, fluorescence excitation, or photonic-resonance-based reflection of liquid samples introduced into the lightpath via custom cartridge. All three modalities utilize off-the-shelf optics mounted in a custom-3D-printed cradle in combination with the rear-facing camera of a smartphone as a high-resolution spectrometer. The use of a bifurcated multimode fiber optic assembly allows for the consolidation of free-space optics into a handheld package while preserving spectral fidelity (pixel resolution = .333 nm/pixel). Each modality is associated with a liquid-handling cartridge that facilitates the appropriate light path and sample-handling requirements, but each of which can be assembled from a common set of laser-cut acrylic and double-sided adhesive components. These cartridges, when coupled with the native video capture capabilities of the smartphone, enable multiplexed video-based data readout of samples via a user-friendly swiping motion through the cradle attachment. The low-cost (< $550) nature of the cradle device and ability to readout three principal modalities of optical biosensing facilitate the straightforward adaption of existing serological assays to smartphone-based readout. For proof-of-concept, we selected two commercial kits and adapted them to the TRI-Analyzer system. In each case, we demonstrate that the new platform is capable of providing equivalent results to those from traditional laboratory spectrometers. Instead of focusing on an assay-specific sensing system, we have demonstrated the strength of this system in its broad flexibility to adapt existing assays to a point-of-care readout.

Keywords: Biosensors, Portable Instruments, Spectroscopy, UV-VIS Absorbance/Luminescence
Application Code: Bioanalytical
Methodology Code: Portable Instruments
Metabolomics enables the identification of putative biomarkers for numerous diseases including cancer; however, the influence of confounding factors on metabolite levels poses a major challenge in moving forward with such metabolites for pre-clinical or clinical applications. To address this challenge, we are employing a well-known econometric method called seemingly unrelated regression (SUR) to analyze metabolomics data from a colorectal cancer (CRC) study and account for the effects of confounding factors such as gender, BMI, age, alcohol use, and smoking. Here, a SUR model based on 113 serum metabolites quantified using targeted tandem mass spectrometry (LC-MS/MS), identified 20 metabolites that differentiated CRC patients, patients with polyps, and healthy subjects. Models built using different groups of biologically related metabolites achieved improved differentiation and were significant for 26 out of 29 groups. Furthermore, the networks of correlated metabolites constructed for all groups of metabolites, before or after application of the SUR model, showed significant alterations for CRC and polyp patients relative to healthy controls. Our results show that demographic covariates, such as gender, BMI, and smoking status, exhibit significant confounding effects on metabolite levels which can be modeled effectively. These results not only provide new insights into addressing the major issue of confounding effects in metabolomics analysis, but also shed light on issues related to establishing reliable biomarkers and the biological connections between them in a complex disease.
Quantitative analysis of protein biomarkers is increasingly using mass spectrometry due to its ability to achieve selectivity coupled with sensitivity. Herein, we developed an LC-MS/MS method for measuring human osteopontin (hOPN) from plasma using immunoaffinity isolations coupled with microflow liquid chromatography tandem mass spectrometry (MFLC-MS/MS). A biologically relevant tryptic peptide GDSVVYGLR which is unique to hOPN was identified and used as a signature peptide for this method. The method was validated over a range of 25-600 ng/mL and applied to healthy and breast cancer samples. In addition, a stable isotope labeled (SIL) peptide GDSVVYGLR* and an extended SIL peptide TYDGRGDSVV*YGLRSKSKKF' were evaluated as internal standards to account for signature peptide digestion instability and variability, and immunocapture variability.

Secondly, biorelevant [i]in vitro[/i] release models are designed to predict the [i]in vivo[/i] behavior of a drug formulation and are crucial in understanding its in vivo performance. Advancement in the biotherapeutics and diversity of parenteral dosage forms have also increased (e.g. ocular implants, drug eluting stents, sinus augmentation implants). A biodegradable locally acting implant that helps in osteoinduction and bone regeneration consisting of two primary components: recombinant human bone morphogenetic protein-2 (rhBMP-2) and an absorbable collagen sponge scaffold for the release of rhBMP-2 over an extended period was used as a model for this second project. Our biorelevant [i]in vitro[/i] drug release (BIVDR) model was designed to correlate to [i]in vivo[/i] pharmacokinetic data and demonstrate that the model is discriminatory between different formulations of rhBMP-2. This [i]in vitro[/i] model is a first-generation release model that could tap into cancer therapeutic release characterization and biomarker studies.
New screening techniques are needed to identify women at increased risk of breast cancer who are the most likely to benefit from more intensive screening programs. Recently, elevated levels of folate-derived pteridines have been reported among women with breast cancer as putative risk stratification biomarkers. However, the biological significance of pteridine metabolites in progressive breast cancer is not fully understood. In this study, we have developed novel metabolic flux analysis techniques to elucidate the pteridine biosynthetic pathway in humans. Pteridine metabolism was studied in the MCF10A isogenic cell line series, which provides a stepwise progression from non-tumorigenic breast epithelial cells toward a malignant phenotype. The detailed experimental procedures, study findings, and conclusions will be presented at the conference. The findings on the biological significance of folate-derived pteridines metabolites in progressive breast cancer make these pteridines potential risk biomarkers for early breast cancer screening, which will ultimately enable clinicians to better understand high-risk populations and improve prevention and detection of breast cancer.

Keywords: Bioanalytical, Biomedical, Chromatography, Mass Spectrometry

Application Code: Biomedical

Methodology Code: Liquid Chromatography/Mass Spectrometry
Recent work on metabolic biomarkers for epithelial cancers has suggested that urinary levels of pteridines and modified nucleosides may be independently associated with increased risk of developing cancer. This study aimed to evaluate whether a novel composite panel of 28 pteridines and modified nucleosides can afford greater accuracy as a risk stratification model. First, an observational case-control clinical study involving 330 post-menopausal women with newly diagnosed breast cancers and matched controls was conducted to optimize the biomarker profile for breast cancer risk assessment. The association between adjusted biomarker levels with breast cancer risk was evaluated using logistic regression models from which odds-ratios and receiver-operation characteristic curves could be computed. Second, the disease specificity of the optimized composite biomarker panel was determined by characterizing its association with several common cancers of epithelial origin, including prostate, bladder, kidney, and lung cancers. Patients with newly diagnosed cancers (N = 130 each cancer) and an equal number of matched controls (650 total study participants) were recruited in a case-consecutive manner across China. Multivariate principal component analyses were used to determine group differences among individual biomarkers to discover possible cancer fingerprinting applications, while group-wise comparisons were made using nonparametric Kruskal-Wallis and Mann-Whitney analyses. Optimized risk stratification models for each cancer type were produced from logistic regression models. Our findings suggest that the combination of urinary pteridines and modified nucleosides can provide improved risk stratification for women with breast cancer and that elevated levels of both biomarker types occur in several common epithelial cancers, which may be differentiated using composite biomarker panels. The detailed findings of the study will be presented at the symposium.

Keywords: Bioanalytical, Biomedical, Clinical Chemistry, Liquid Chromatography/Mass Spectroscopy
Application Code: Biomedical
Methodology Code: Liquid Chromatography/Mass Spectrometry
A Closer Look at Clinical Toxicology Screening in Different Matrices

Breath Gas Monitoring of Drug Metabolites in Real Time - PTR-ToF-MS Analysis of Prilocaine Concentrations by o-toluidine

Real time breath gas monitoring by hyphenated MS-techniques opens new perspectives for applications in the clinical field. A rare but potentially life-threatening side effect in the use of the local anaesthetic prilocaine (N-(2-Methylphenyl)-2-(propylamino)-propanamid) is the generation of methaemoglobin. The diagnosis is currently only possible by means of blood tests. Especially for risk patients rapid and non-invasive tests would be desirable.

Using a pig model, we induced methaemoglobinemia via application of dimethyl aminophenol and prilocaine or natrium nitrite and prilocaine. Continuous real time breath gas monitoring for non-invasive determination of breath VOCs was performed by PTR-ToF-MS. The concentrations of the main metabolite o-toluidine were quantified in the alveolar breath. O-toluidine could be determined with excellent linearity (R2=0.99) in concentrations up to 100 ppbV. Limits of detection and quantification were 0.09 ppbV and 0.23 ppbV, respectively. The o-toluidine concentrations determined in pigs’ breath by means of PTR-ToF-MS were in the range of 0-23.5 ppbV. The administration of prilocaine intravenously was clearly reflected with a time lag of a few minutes by the detection of o-toluidine in the respiratory gas. Repetitive injections of prilocaine induced consecutive increases in breath o-toluidine concentrations.

PTR-ToF-MS enables continuous real time determination of o-toluidine in breath gas after intravenous administration of prilocaine. In a perspective, detection of prilocaine induced methaemoglobinemia and avoiding of related hypoxia might be possible by non-invasive breath tests.

Keywords: Clinical/Toxicology, Mass Spectrometry, Time of Flight MS, Volatile Organic Compounds
Application Code: Clinical/Toxicology
Methodology Code: Mass Spectrometry
A Closer Look at Clinical Toxicology Screening in Different Matrices

A Quantitation Method of Serum Testosterone by LC-MS/MS with Online Extraction

Background: Measurement of testosterone in circulation is important in adult and pediatric endocrinology. The most commonly employed methods in the clinical laboratories are antibody-based immunoassays. However, these assays do not have the sufficient sensitivity and specificity to measure low levels of serum testosterone, especially in women, children, and men with androgen suppression therapies. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) has been increasingly used in serum testosterone measurements that require high sensitivity. We have developed an LC-MS/MS method for serum testosterone measurement with online sample extraction.

Methods: Serum sample (200 μL) was mixed with 13C3Testosterone as internal standard in 400 μL acetonitrile, and spun at 4000 xg. The supernatant (50 μL) was injected into online C18 trap column followed a HPLC system coupled with a QTRAP 5500 mass spectrometer. The mass spectrometer was set at positive ESI and multiple reaction monitoring. The MRM transitions used for the testosterone were 289.2→97.2, 289.2→109.2, and for the 13C3Testosterone IS, 292.2→100.2 and 292.2→112.2.

Results: The limit of quantification was 20 pg/mL and the imprecision was found to be <15% CV at the limit of quantitation and <7% CV at higher concentrations. Epi-Testosterone, androstenedione(AD), and dehydroepiandrosterone(DHEA), were baseline separated from testosterone a 5-min run-time.

Conclusions: This LC-MS/MS method is sufficiently sensitive and specific for measuring serum testosterone that requires high sensitivity. It requires little manual sample preparation due to efficient online extraction.

Abstract Text

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Keywords: Clinical Chemistry, Clinical/Toxicology, Liquid Chromatography/Mass Spectroscopy, Validation
Application Code: Clinical/Toxicology
Methodology Code: Liquid Chromatography/Mass Spectrometry
Some 476,000 military personnel with post-traumatic stress disorder (PTSD) have developed drug and/or alcohol dependence. Buprenorphine is becoming the medication of choice to help veteran and non-veteran patients alike withdraw from opioid addiction. Veterans Affairs hospital physicians must frequently test patients to identify the discontinuation of medications or any recurrence of drug use and then adjust treatment appropriately. These tests most often involve collecting and sending a urine sample to a clinical lab for analysis by gas chromatography coupled mass spectrometers. Results are usually returned in 1-2 weeks, a delay that makes timely adjustment of treatment difficult. Here we describe the development of a point-of-care (POC) analyzer that can measure both illicit and treatment drugs in patient saliva, ideally in the physician’s office within 10 minutes. The analyzer employs a relatively simple supported liquid extraction to isolate the drugs from the saliva and surface-enhanced Raman spectroscopy (SERS) to detect the drugs. The SERS-based POC was used to measure opioids and buprenorphine in saliva samples collected from veterans. In addition, acetaminophen, caffeine, delta-9 THC, and nicotine were detected in several samples. Details of the methodology and results will be presented.
Glucose incorporation in tissues is enhanced through exercise regimes; however, the metabolic interactions between tissues are limited in the literature. Our analysis of an exercise state infers metabolites are involved in extensive recycling to both small molecules and complex lipids, and indicate small molecules that are not depleted throughout the process. Our experiment interprets metabolic networks from exercise by analyzing plasma, gastrocnemius muscle and heart tissue of C57BL/6J and BALB/c mice exercised at various conditions in response to applying a uniformly labeled 13C (U13C-labeled) glucose intraperitoneal bolus injection prior to exercise as a tracer. Lipids were extracted using the traditional Folch method, whereas metabolites were extracted in acetonitrile:methanol:acetone (8:1:1, v:v:v). Data were collected on a Dionex liquid chromatography system integrated with a Thermo Orbitrap Q-Exactive mass spectrometer. Preliminary data indicate dynamic rearrangements of glucose towards the glycerol backbone of specific lipid biomarkers such as triacylglycerides and cardiolipin. If due to re-esterification, this could include tissue-tissue interactions suggesting dynamic flux interchange between tissues with exercise. Additional studies are evaluating U13C incorporation from glucose to carnitines, to further identify significant metabolites that are markers of exercise metabolism. Additionally, this study intends on evaluating the pathways impacted by exercise that can be detected with 13C glucose as a tracer.

Keywords: Biomedical, Lipids, Liquid Chromatography/Mass Spectroscopy, Metabolomics
Application Code: Clinical/Toxicology
Methodology Code: Liquid Chromatography/Mass Spectrometry
Base oils are a vital component in the manufacture of lubricating greases, motor oils and metal processing fluids. Produced by refining crude oil or through chemical synthesis, the American Petroleum Institute classifies these oils into five groups. Groups I-III consist of petroleum-based hydrocarbons derived from crude oil. Group IV consist of fully synthetic poly-alpha-olefins (PAOs), while group V consist of all other base oils that do not fall in the previous groups. With a vast range of boiling points and carbon numbers, the task of analyzing such a complex mixture is very difficult. The need to monitor industrial petroleum processing steps as well as screen and identify petrochemical environmental pollutants drives the necessity for an analytical method that surpasses traditional one-dimensional gas chromatography (GC). Comprehensive two-dimensional gas chromatography (GCxGC), an analytical technique that employs two columns connected by a modulating interface, allows for improved resolution, increased peak capacity and higher sensitivity when compared to 1D GC. GCxGC with a reverse fill/flush flow modulator and time-of-flight mass spectrometer (TOF-MS) were utilized to achieve separation of several conventional and synthetic motor oils. A reverse column set, polar first dimension and apolar second dimension, allowed for superior group type separation of all classes, including linear, branched and aromatic species. Chemical fingerprinting and statistical comparisons allowed for the differentiation of motor oil type and manufacturer. The results show the ability to achieve a comprehensive separation and identification of specific classes of compounds within a complex mixture in a single analysis.

Keywords: Fuels\Energy\Petrochemical, Gas Chromatography, Gas Chromatography/Mass Spectrometry, Petrol
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Gas Chromatography/Mass Spectrometry
Peak tailing and internal standard reproducibility often make quantitative analysis of polyaromatic hydrocarbons (PAHs) particularly challenging. The addition of a complex matrix only makes experiments even more problematic. These difficulties are inherent to characterizing PAH levels in samples of used engine oils, which not only gives insight into engine combustion efficiencies but also helps ensure proper environmentally-conscious waste disposal. Using a variety of techniques including comprehensive two-dimensional gas chromatography (GC×GC) and better-than-nominal resolution time-of-flight mass spectrometry (TOFMS), PAHs are separated from matrix interferences using selectivity in both orthogonality in column phases and mass accuracy. Identification of specific compounds is accomplished by retention time correlation with standard mixes and full-mass range spectral matching with commercial libraries, while calibration curves are built with the flexibility to choose multiple quantitation methods and the option to normalize internal standard responses. With common quantitation challenges overcome, relative PAH levels in used engine oils are compared between gasoline-powered engines in cars that routinely travel short and long distances. A direct comparison of the ratios between PAHs and their methylated counterparts provide insight into the nature of combustion by-products that occur when engines are routinely operated under different conditions.
The quantitation of olefins in petroleum products is of great interest to the industry, especially for those involved in transportation and upgrading of bitumen and heavier crudes by thermal cracking (visbreaking). Olefins and di-olefins produced in small quantity during the thermal upgrading processes have a tendency to undergo polymerization reactions causing instability to the finished products.

The purpose of this research project was to identify and quantify olefins using Gas Chromatography (GC) and a Vacuum Ultraviolet (VUV) detector. The focus matrices were condensates and upgraded bitumen.

The research was successful and has resulted in the identification and quantitation of more than 60 olefins and diolefins including several conjugated olefins. Method detection limits (MDLs) at very low part per million (ppm) levels with greater than 90 percent accuracy were achieved.

This presentation will discuss the research, analytical parameters such as precision, detection limits, method dynamic range and accuracy, and the results from sample data. The theory and the function of the detector will also be discussed.
Thermal cracking/conversion of heavy oils and bitumen is an important upgrading and refining process but, during which, olefins and diolefins are generated. These compounds are perceived to be problematic, as they cause instability and fouling issues in downstream refining. Presently there are limited options for the separation and characterization of these compounds, and methods for accurate quantification are needed. This presentation describes a new method that uses normal phase high performance liquid chromatography (HPLC) to separate olefins and diolefins in petroleum streams. A silver ion column was used to achieve the separation, and quantification of both model compounds and real samples and separation data will be shown. Challenges encountered during method development will be discussed, along with their solutions. HPLC results will be compared to other olefin measurement techniques to demonstrate both advantages and disadvantages. This method will help improve our understanding of thermal conversion process chemistry and aid in the development of technologies for removing olefins/diolefins in thermally cracked products, reducing or eliminating associated problems. Funding for this work was provided by the Government of Canada’s interdepartmental Program of Energy Research and Development (PERD).
Advances in Fuels, Energy, and Petrochemical Analysis

Rhodamine GUMBOS: Improved Energy Relay Dyes for Dye-Sensitized Solar Cells

Dye-sensitized solar cells (DSSCs) are of interest because they 1) work in low solar irradiation environments, 2) can be constructed to be flexible for use in backpacks, and 3) can be used in inconspicuous environments such as window décor. However, in order for DSSCs to be competitive with current technology, overall efficiencies of these systems must be improved. The photosensitizing dye component in DSSCs plays a critical role by absorbing photons from sunlight and transferring electrons to generate current. However, use of a second dye dissolved in the electrolyte, known as an energy relay dye (ERD), has been shown to further enhance solar conversion efficiency. In this talk, we examine a new class of materials known as a Group of Uniform Materials Based on Organic Salts (GUMBOS) as ERDs. GUMBOS are solid phase organic salts composed of bulky organic ions with melting points in the range of 25°C to 250°C. The properties of GUMBOS have been shown to be tunable through variations in the counter-ion, resulting in a unique material possessing characteristics different from the parent ions. We have shown that by use of counter-ions commonly employed in ionic liquids, we can improve the solubility of GUMBOS in the electrolyte, as well as provide better flexibility in optical properties. In this study, GUMBOS are synthesized using rhodamine dyes that have a variety of ligands attached to the cation. Studies of the optical properties of these compounds using ultraviolet-visible absorbance spectroscopy and fluorescence spectroscopy were performed. Solar efficiencies were evaluated in the absence and presence of ERDs using N719 as the photosensitizing dye. DSSCs in the presence of GUMBOS-based ERDs were found to have increased solar efficiencies as compared to those without ERDs. These studies give further insight into the importance of the counter-ion used in GUMBOS synthesis and its relationships to a change in solar efficiency.

National Science Foundation CHE-1307611

Abstract Text
Dye-sensitized solar cells (DSSCs) are of interest because they 1) work in low solar irradiation environments, 2) can be constructed to be flexible for use in backpacks, and 3) can be used in inconspicuous environments such as window décor. However, in order for DSSCs to be competitive with current technology, overall efficiencies of these systems must be improved. The photosensitizing dye component in DSSCs plays a critical role by absorbing photons from sunlight and transferring electrons to generate current. However, use of a second dye dissolved in the electrolyte, known as an energy relay dye (ERD), has been shown to further enhance solar conversion efficiency. In this talk, we examine a new class of materials known as a Group of Uniform Materials Based on Organic Salts (GUMBOS) as ERDs. GUMBOS are solid phase organic salts composed of bulky organic ions with melting points in the range of 25°C to 250°C. The properties of GUMBOS have been shown to be tunable through variations in the counter-ion, resulting in a unique material possessing characteristics different from the parent ions. We have shown that by use of counter-ions commonly employed in ionic liquids, we can improve the solubility of GUMBOS in the electrolyte, as well as provide better flexibility in optical properties. In this study, GUMBOS are synthesized using rhodamine dyes that have a variety of ligands attached to the cation. Studies of the optical properties of these compounds using ultraviolet-visible absorbance spectroscopy and fluorescence spectroscopy were performed. Solar efficiencies were evaluated in the absence and presence of ERDs using N719 as the photosensitizing dye. DSSCs in the presence of GUMBOS-based ERDs were found to have increased solar efficiencies as compared to those without ERDs. These studies give further insight into the importance of the counter-ion used in GUMBOS synthesis and its relationships to a change in solar efficiency.

National Science Foundation CHE-1307611

Keywords: Electrochemistry, Fluorescence, Fuels\Energy\Petrochemical, UV-VIS Absorbance/Luminescence
Application Code: Fuels, Energy and Petrochemical
Methodology Code: UV/VIS
Characterization of Intermetallic Structures Towards the Chemoselective Hydrogenation of Nitroarenes to Functionalized Anilines

Molecular engineering of catalytic surface is critical to understand and to design heterogeneous catalysts satisfying high selectivity and activity. To address these desires in bimetallic systems, it is urgent to precisely control the bimetallic surface and to correlate such surface to specific catalytic pathways. Different from random alloys, intermetallic compounds (IMCs) present atomically-ordered structures, which is advantageous for catalytic mechanism studies. To overcome the aggregation during the high temperature annealing to prepare intermetallic nanoparticles (iNPs), we developed a seeded growth strategy to prepare a series of Pt-based alloy/iNPs encapsulated in mesoporous silica shells (PtM, Pt3M@mSiO2, M=Sn, Zn, Pb, Co, Ni, Cu and Pd). These iNPs have tunable composition with clean surfaces and homogenous structures. We performed a variety of advanced characterization to investigate the structural and surface information of these iNPs, including HAADF-STEM, AP-XPS, XPS, PXRD, CO-DRIFTS and ICP-MS. Our iNPs demonstrate a well-ordered and thermostable intermetallic structures. Chemoselective hydrogenation of 3-nitrostyrene was studied over PtSn, Pt3Sn iNPs and Pt NPs. PtSn iNPs shows >99% selectivity to hydrogenate the nitro group of 3-nitrostyrene and other various nitroarenes. Further kinetic studies demonstrate a geometric effect that catalytic pathways can be readily altered upon different intermetallic structures. This study emphasizes the efforts to clarify the surface structures of IMCs to establish a critical relationship between IMCs and their catalytic properties.

Keywords: Characterization, FTIR, Surface Analysis
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Surface Analysis/Imaging
Gold nanorods show great potential in harvesting natural sunlight and generating hot charge carriers that can be employed to produce electrical or chemical energies. We show that photochemical reduction of Pt(IV) to Pt metal mainly takes place at the ends of gold nanorods (AuNRs), suggesting photon-induced hot electrons are localized in a time-averaged manner at AuNR ends. To use these hot electrons efficiently, a novel synthetic method to selectively overgrow Pt at the ends of AuNRs has been developed. These Pt-end-capped AuNRs show relatively high activity for the production of hydrogen gas using artificial white light, natural sun light, and more importantly, near IR light at 976 nm. Tuning of the surface plasmon resonance (SPR) wavelength of AuNRs changes the hydrogen gas production rate, indicating that SPR is involved in hot electron generation and photo-reduction of hydrogen ions. In addition, these Pt-end-capped AuNRs are used in combination with a ferroelectric material. Our preliminary data shows that this composite particle catalyst can effectively extend the lifetime of the charge carriers generated on Pt-end-capped-AuNRs in the presence of the ferroelectric materials, leading to a greater production efficiency of hydrogen gas. This study shows that gold nanorods are excellent photocatalysts for converting low energy photons into high energy hot electrons.

**Keywords:** Fuels/Energy/Petrochemical, Materials Characterization, Nanotechnology, Near Infrared

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

**Methodology Code:** Near Infrared
Perfluorinated compounds have been synthetically produced since the 1950s but have only recently been linked to diseases such as chronic kidney disease. One of the most prevalent of these compounds is perfluorooctanesulfonic acid (PFOS). This compound has been found in ng/mL concentrations in human blood and was detected in 99 % of individuals tested in a 2008 study. These findings have led to a large number of studies to determine the best methods to prevent exposure to, contain, and/or clean up PFOS. One promising method to contain PFOS contaminations has been the use of polyelectrolytes. These polyelectrolytes can be used as barriers to strongly bind PFOS, stopping its transport within groundwater. Currently, polyelectrolytes have been shown to strongly bind alkyl-based surfactants However, the binding of perfluoroalkyl surfactants for polyelectrolytes has not been reported limiting their use as barriers. Therefore, we implemented fluorous-phase ion-selective electrodes to provide quantitative measurements of the binding of PFOS to polyelectrolytes.[superscript 1] These fluorous-phase ion-selective electrodes were developed using a new ion exchanger based on a perfluorinated imidazolium derivative. These electrodes were used to measure the cooperative binding of PFOS to two polyelectrolytes (polydiallyldimethylammonium) and a copolymer of epichlorohydrine and dimethylamine, providing evidence for their use as barriers for PFOS.

1. [i]Anal. Chem.[/i] [b]2013[/b], [i]85[/i], 7471-7477.

Keywords: Contamination, Electrochemistry, Ion Selective Electrodes, Polymers & Plastics

Application Code: Environmental

Methodology Code: Electrochemistry
Heavy metals have been a severe environmental problem that has gained momentum due to increased public exposure. Recent events with Pb and Mn exposure have drawn significant attention as a neurotoxin in children with chronic exposure. Thus, a rapid, reliable method is required to analyze these metals in environmental and biological samples. Even though, traditional methods (eg: spectroscopy) provide sensitive reliable data, they are costly and time consuming making them unfeasible for point-of-care (POC) detection. Yet, the commercially available POC systems only provide single metal detection or require larger sample volumes. Here, we describe a potential POC system via stripping voltammetry that could detect both Pb and Mn simultaneously using a small sample volume (eg: 10 µL). Anodic stripping voltammetry is optimized for detection of Pb with a LOD of 5 ppb via a gold working electrode, while Mn is detected using cathodic stripping voltammetry with a LOD of 1 ppb via a platinum working electrode. The system is validated with environmental water samples with >90% accuracy and >97% precision for both Pb and Mn concentrations as compared with ICP-MS gold standards measurements. The high accuracy and precision of our technique confirms that our system can be effectively used as a POC system to detect Pb and Mn in clinical blood samples.

**Keywords:** Electrochemistry, Environmental/Biological Samples, Sensors, Stripping Analysis

**Application Code:** Environmental

**Methodology Code:** Electrochemistry
We report the fabrication of a 3D surface enhanced Raman scattering (SERS) substrates composed of silver coated gold nanorods (Ag/AuNRs) supported on polycaprolactone (PCL) electrospun fibers following the fabrication protocol developed in our previous study for gold nanorods (AuNRs). The successful fabrication of Ag/AuNRs onto PCL fibers demonstrated the universal nature of our fabrication strategy, benefiting from immobilization based on electrostatic attraction, a non-specific interaction, as the driving force. Further, Ag/AuNRs with different Ag coating thicknesses have been synthesized, characterized and assembled onto PCL fibers to obtain Ag/AuNRs based SERS substrates respectively. By using 4-Mercaptopyridine (4-Mpy) as a probe molecule, the SERS performance of the Ag/AuNRs based SERS substrate has been investigated. This sensitive substrate allowed a detection of 4-Mpy at a concentration as low as 10^-8 M, and its excellent reproducibility was also demonstrated. SERS substrates fabricated with Ag/AuNRs with different Ag coating thicknesses and uncoated AuNRs have been compared. It was found that when fabricated with a Ag coating, the substrates exhibited larger SERS enhancement than that found for substrates fabricated with AuNRs, and this could be attributed to electron transfer between the gold core and silver shell in a bimetallic rod-shape nanostructure. By utilizing the available surface chemistry provided by the silver coating, arsenic species could be detected. Four arsenic species were studied, including two organic arsenic compounds as veterinary antimicrobials (p-arsanilic acid and roxarsone) and two inorganic compounds (arsenite and arsenate) which are the most common arsenic species found in water. Calibration and quantification studies of all these four compounds have been carried out on these SERS substrates and a detection limit of 10 ppb (guideline limit concentration set by the World Health Organization) was achieved.
Development of a Self-Cleaning Boron-doped Diamond Sensor for Continuous Quality Monitoring in Drinking Water Distribution Systems

Long-term, continuous monitoring with electrochemical sensors in complex media is challenging due to the effects of biofouling. Drinking water biofilms reduce sensor performance by inhibiting mass transport of analytes to sensor surfaces. Boron-doped diamond (BDD) electrodes are more resistant to biofouling than conventional electrodes, and remain inert at high potentials. These properties can be exploited to produce a sensor that can resist biofouling, and electrochemically remove biofilm on sensor surfaces using high potentials, without disassembly or disconnection. Incorporating BDD into a wall-jet flow cell, a geometry that is highly scalable with minimal dead volume, has resulted in the production of prototype self-cleaning sensors which have been tested in both the laboratory and in a drinking water treatment works. Prototype development started with commercially available sensors which are intended for use with filtered and degassed solutions and at stable laboratory conditions. Regular cleaning and polishing prevent these sensors encountering the difficulties of continuous monitoring in complex media, including biofouling. We have demonstrated that a BDD, wall-jet flow cell is both able to detect free chlorine accurately, with a standard error of 4.86%, and with a limit of detection which is less than the accepted minimum required concentration of 0.2 ppm. Additionally, the prototype sensor was capable of detecting the presence of a drinking water biofilm indicator organism, P. aeruginosa. The in situ cleaning process also compares very favourably to manual sensor surface cleaning methods.

After testing earlier versions in both the laboratory and in the field, new prototype sensors are being manufactured. These sensors will be installed in drinking water distribution systems to assess long-term performance of free chlorine detection in a challenging environmental matrix.

Keywords: Analysis, Electrochemistry, Sensors, Water
Application Code: Environmental
Methodology Code: Electrochemistry
Ammonia is a common naturally occurring substance, also manufactured by man. At normal environmental conditions, pure ammonia is a colorless, pungent-smelling and corrosive gas. It is highly soluble in water and reacts with acids to form ammonium salts. Ammonia is used in a variety of ways: for bleaching or cleaning, in the production of fertilizers, plastics, pharmaceuticals, rubber and petrochemicals and as an anti-fungal agent for foodstuffs. The main sources of ammonia are from decaying organic matter, the excreta of humans and animals, the use of fertilizers, waste disposal sites and industrial processes.

The main local problem of ammonia is the unpleasant odor, which is detectable even at low concentrations. At high concentrations it can also harm vegetation. The harm caused by ammonia in water bodies is more serious, since it is very toxic to aquatic organisms. Low concentrations of ammonia in soil are natural and actually essential for plant nutrition. Ammonia is also a precursor for particles.

European laws control ammonia releases to air and water; releases from industrial plants (84/360/EEC); and ammonia pollution of the aquatic environment (76/464). UK releases of ammonia are controlled under the Pollution Prevention and Control (PPC) regulations; and regulations on releases to surface waters.

We address the low level ambient ammonia monitoring need with tunable quantum cascade laser combined with patented cantilever enhanced photoacoustic detection [1,2]. Ultimately sub-ppb level detection limits are demonstrated, which proves high suitability for the purpose of continuous ammonia monitoring for leak detection, fugitive emissions and ambient air quality.


Keywords: Agricultural, Environmental/Air, Molecular Spectroscopy, Photoacoustic

Application Code: Environmental

Methodology Code: Molecular Spectroscopy
Magnetic biochar was successfully prepared for quick removal of nitrate and fluoride ions from aqueous solutions. This study highlights the conversion of Douglas Fir biochar into magnetic biochar. Magnetic biochar was obtained by pyrolyzing FeCl₃ soaked Douglas Fir biochar at 600 °C with a residence time of 1h under N₂ gas. The characterization of both magnetized and non-magnetized biochars were studied using scanning electron microscopy (SEM), scanning electron microscopy-energy dispersive X-ray spectroscopy (SEM-EDX), transmission electron microscopy (TEM), surface area measurement (S BET), point of zero charge (PZC) measurements, X-ray diffractometry (XRD) analysis, hysteresis and magnetic moment measurements, and X-ray photoelectron spectroscopy (XPS). Characterization results show the successful magnetization of biochar with Fe₂O₃ particles. Batch sorption studies were performed at pH values from 2 to 11, and temperatures from 298 to 318 K. The total amount of nitrate adsorbed was determined quantitatively using liquid chromatography using an ultraviolet detector (LC-UV) and the total amount of fluoride adsorbed was determined quantitatively using UV-Vis double beam spectrometry with SPADNS reagent at 580 nm. The magnetized biochar showed enhanced removal of nitrate and fluoride from solution compared to non-magnetized biochar, suggesting that the modification of biomass with FeCl₃ generates different adsorption sites for nitrate and fluoride ions adsorption. Both nitrate and fluoride adsorption on modified biochar remained unchanged with a rise of solution pH from 2-10. Sorption was evaluated from 298 to 318 K using the Langmuir and Freundlich isotherm models and Langmuir adsorption capacities as high as 15.5 mgg⁻¹ for nitrate and 9.04 mgg⁻¹ for fluoride were observed. This represents a much higher capacity than the reported values of other adsorbents.

Keywords: Adsorption, Environmental/Waste/Sludge, Environmental/Water, Surface Analysis
Application Code: Environmental
Methodology Code: Process Analytical Techniques
Development of systems for capture, sequestration and tracking of nanomaterials is becoming a significant focus in many aspects of nanotechnology and environmental research. Such measurements are critical for evaluating concentration, distribution and effects of nanoparticles (NPs) for environmental, clinical, epidemiological and occupational exposure studies. This presentation will describe a portable paper-based tracking system that can simultaneously capture and quantify metal oxide NPs in complex environments. The approach involves the use of capture ligands containing ortho-dihydroxy functionality, grafted on filter paper, enabling multivalent binding and formation of strong charge transfer complexes with the NPs. The distinct binding of the NPs to multidentate ligands coupled with the spectral properties of the resulting charge transfer complexes enabled quantitative detection of CeO2 NPs in a concentration-dependent manner and revealed good sensitivity with detectable concentration in the 10^11 NP/mL range and excellent reproducibility. The binding also produces visual images of the particle content and distribution which provides capabilities for a broad range of applications for separation, characterization and quantification of NPs in a variety of environments. These results can enable further development of devices and separation technologies including platforms for retention of NPs and measurement tools for detection and differentiation of metal oxide NPs based on differences in catalytic reactivity and surface functionality. Development of methodologies for assessing workers exposure to NPs and for measuring presence of NPs in environmental or biological samples could also be envisioned.
Carbon quantum dots (CQDs) have emerged out as potential competitor to semiconductor QDs to develop an optical sensor platform, and are defined as quasi-spherical nanoparticles consisting of amorphous to nano-crystalline cores. Their excellent features in terms of high chemical stability, low cost synthesis, easy scalability, environmental friendliness, broadband optical absorption, low toxicity, strong photoluminescence (PL) emission, and optical properties make them a potential material in photo-catalysis, bio-sensing, drug delivery, chemical sensing, bio imaging and electro-catalysis domain. Herein, we present them as sensitive and selective sensor material for toxic heavy metals & metalloids (Arsenic, Selenium & Chromium) detection in environmental water below WHO guidelines. The surface of as synthesized CQDs is modified with appropriate ligand to impart selectivity and sensitive assays are established for their application in toxic metals/metalloids determination in water. The developed sensor interface is fully characterized in terms of its structural (HR-TEM, XRD, SEM, etc.) and optical (UFS, XPS, PL, UV-Vis, Raman) properties. Besides, the technical parameters have been extracted to design a portable optical system for on-site detection of these pollutants in water, which is the present need. The in-vitro toxicity study of CQDs is also conducted to understand their toxic effect before environmental application deployment. The present work in summary utilizes the material science and fluorescence spectroscopy to develop a portable optical system for water pollutants (arsenic, chromium and selenium) monitoring in water.
Comprehensive two-dimensional gas chromatography (GC×GC) is a powerful technique for the separation of complex mixtures, providing a significant increase in selectivity and peak capacity. Since its inception, the focus of research and development in GC×GC has been primarily the modulator design. While it has continually evolved, improvements to second dimension separation have largely been ignored. Optimization of a GC×GC system is often complicated due to the coupling of the primary and secondary columns within a shared GC oven. Furthermore, additional problems like peak wraparound need to be eliminated or minimized. Wraparound peaks eluting in subsequent modulation cycles potentially co-elute with separated compounds. The use of a secondary oven is often the solution to alleviate this problem. By providing a constant positive temperature offset from the main GC oven temperature, retention of all analytes can be reduced so that they elute within a single modulation period. However, because of the practically isothermal conditions during the limited second dimension separation time, the classical general elution problem leads to loss of separation between less retained analytes. To overcome this problem, the second dimension in GC×GC was temperature programmed by resistively heating an electrically conductive secondary column. Cooling was accomplished through forced convection inside the GC oven within the time frame of a modulation period. Temperature programming in the second dimension of GC×GC was able to eliminate peak wraparounds and reduce peak widths, leading to increased second dimension peak capacity.
Ionic Liquids (ILs) are state-of-the-art fluids that have found broad application in separation science. Owing to their unique physicochemical properties such as low vapor pressure, high thermal stability, and great viscosity, they were successfully used as stationary phase in gas chromatography (GC). ILs are considered as polar GC stationary phases with polarity ranging from a McReynolds constant of 2666 for SLB-IL60 to the most polar SLB-IL111 with a McReynolds constant of 5150. By properly engineering their molecular structure, ionic liquids with tailor-made properties can be designed. Therefore, a systematic structure-function relationship can be established to study the effect of structural modification on the properties of ionic liquids. Herein, we present designing of dicationic ionic liquids with unique retention characteristics when used as GC stationary phase. The main goal of this study is to make extremely polar ILs by manipulating the cationic moiety, spacer chain, or counteranions. Different methods were developed to assess the selectivity and polarity of new IL phases. The results were compared with SLB-IL111, the most polar commercially available GC column. Finally, their capabilities to separate cis and trans fatty acid methyl esters (FAMEs), and polycyclic aromatic hydrocarbons (PAHs) were evaluated.

Keywords: Forensics, Gas Chromatography, GC Columns, PAH
Application Code: Process Analytical Chemistry
Methodology Code: Gas Chromatography
Our recently introduced modeling software is a selectivity tool that relies on a pre-loaded library of thermodynamic retention indices. This makes it possible to predict retention times and optimize chromatographic methods without the need to analyze compound sets under many different conditions. The program allows the user to select the stationary phase and simultaneously adjust: film thickness, temperature, column length, column internal diameter and flow. Users can enter each compound or cut/paste large lists of compounds into the program.

Since its introduction there have been thousands of searches across a broad range of compound classes. The program outputs: compound retention time, resolution and peak width along with the column conditions and dimensions. A model chromatogram is provided to illustrate retention, peak width and resolution. Users have the option to view compound mass spectral data with the added benefit of overlaying mass spectra for coeluting analytes. Specific searches can be saved and accessed at a later date. Examples of these features will be presented with a focus on challenging separations.

Keywords: Capillary GC, Chromatography, High Throughput Chemical Analysis
Application Code: High-Throughput Chemical Analysis
Methodology Code: Gas Chromatography
Sediment material from corrosion (containing magnetic iron (oxide based)) has been found accumulating in pumps, valves, on pigs and in line inspection tools. In more viscous streams, the solids may be suspended in the stream and not result in the same operational issues, however they may cause issues in tankage with settling. Condensates are low viscosity and have limited ability for solids retention so the issue may appear more readily in these lines.

Some of the key players in corrosion are oxygen (O2), carbon dioxide (CO2) and hydrogen sulfide (H2S). These can cause corrosion when present in the low ppm to ppb range. At this time, a robust, reliable method to detect trace levels of dissolved corrosion gases (O2, H2S and CO2) in pipeline products does not exist. Headspace gas chromatography (GC) and gas oil ratio (GOR) techniques have previously been considered and/or evaluated, however they will not work for the trace amounts of dissolved gas that are to be detected.

A method was developed that uses a liquid phase injection, a backflush so the fixed gas column does not get contaminated, and a sensitive detector. Low ppm detection limits were achieved.

**Keywords:** Fuels\Energy\Petrochemical, Gas Chromatography, Method Development, Sample Introduction

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

**Methodology Code:** Gas Chromatography
There has been considerable interest in the development of microfabricated silicon columns for gas chromatographic separations. These columns are attractive since they allow rapid temperature programming under low power conditions, and these devices can be produced at reduced cost as they can be manufactured by batch fabrication techniques. Recently, we have reported the development of high performance microfabricated separation columns by utilizing room temperature ionic liquids (RTILs) as the stationary phase materials. Herein, we evaluated the effect of separation performance of these columns by immobilizing an RTIL on alumina surface, which was coated on the silicon surface by atomic layer deposition. A 1 m long, 240 μm deep, 190 μm wide column with embedded circular micropillars was first coated with alumina followed by deposition of an RTIL film. The column was then tested for gas chromatographic separation of two different mixtures: a 21-component mixture of hazardous chemical compounds and a diesel sample. A significant improvement in separation efficiency was observed on depositing RTILs on alumina surface as compared to the columns prepared by depositing an RTIL on silicon surface. These studies highlight the feasibility of improving the separation performance by optimal selection of surface prior to immobilizing RTILs.

Keywords: Environmental, Gas Chromatography, GC Columns
Application Code: Environmental
Methodology Code: Gas Chromatography
**AIM**: To develop a complete lab-on-a-chip (LOC) system for gas-chromatographic analyses to reach the best performances in terms of sensitivity, linearity and response time for natural gas industry applications.

**METHODS**: Micro-Electro-Mechanical Systems (MEMS) are one of the most promising technologies for the 21st Century. These have allowed the miniaturized fabrication of the complete analytical core of the microGC system developed, with the following specific characteristics:

1) separation columns, with unique circular cross section with diameters ranging from hundreds to tens of microns, based on a sequence of anisotropic-isotropic etchings on silicon wafers
2) non-traditional microfluidic interconnections between the three MEMS-based main components of the microGC analytical core
3) with appropriate modifications, possibility to perform experimental tests with isothermal or temperature/pressure programming configuration

**RESULTS**: The first results have been obtained with the isothermal configuration for natural gas heating value calculations. Sampling frequency was tuned up to 1 kHz, allowing extremely fast data acquisition rate. The analysis time were below 5 minutes, and the sensitivity for the n-Hexane was 0.2ppm instead of 0.5-1ppm of current microGC. In addition, the carrier gas flow and the energy consumption were significantly reduced due to the avoidance of conventional microfluidic interconnections and the use of microscopic MEMS, making this robust technology ideal to be used also in remote areas.

**CONCLUSIONS**: The fully MEMS-based microGC has been tested for the first time avoiding traditional fused-silica interconnections. The obtained results confirm that this is the most promising and cost-effective technology for natural gas analyses for the next years.

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

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

**Methodology Code**: Gas Chromatography
We report on a new collector module for a microscale gas chromatograph (µGC). This Si-microfabricated, ultra-low-power micro-collector/injector (µCOIN) combines a passive preconcentrator (µPP) with a progressively heated injector (µPHI). The µCOIN is designed to sample airborne vapors at known rates over a wide range of concentrations and durations by diffusion (no pump) and then inject them to a downstream µGC under active flow. This talk concerns the µPP. Each µPP device (8 x 8 mm) has a grid of precision-etched access holes in the top layer and two concentric adsorbent-retention cavities with integrated thin film heaters in the bottom layer. By varying the number and size of the access holes we have created a high-rate µPP for short-term (e.g., 15-30 min) sampling and a low-rate µPP for 24-hr sampling. Prior to testing, thermogravimetric analysis was performed to assess the adsorption capacities of each of 4 vapors at relevant concentrations for the graphitized carbon adsorbents, Carbopack X (C-X) and Carbopack B (C-B). We found that capacities ranged from 4623 to 46479 µg/g and varied linearly with $p^{-1}$. For m-xylene, the capacity followed a Langmuir isotherm. In all cases, capacities were sufficient to ensure proper device operation using 400 mg of C-X and 800 mg of C-B loaded into the inner and outer cavities, respectively. Performance testing to date showed that the high-rate device sampling rate is lower than model predictions, but constant for 30 min at a challenge of 8 ppm. The thermal-desorption transfer efficiency was > 95% (250 °C) at flow rates compatible with µGC separations, and performance was reproducible (RSD < 5% for n = 3). On-going testing will assess the constancy of the sampling rate as a function of time, concentration, and the presence of competing vapors for both the high-rate and low-rate µPP devices.

**Keywords:** Gas Chromatography, Lab-on-a-Chip/Microfluidics, Sampling, Volatile Organic Compounds

**Application Code:** Environmental

**Methodology Code:** Gas Chromatography
Thermal gradient gas chromatography (TGGC) differs from commonly used temperature programmed gas chromatography (TPGC) in that the column temperature decreases from the injector to the detector, which allows for focusing of the analyte bands as they move through the column. Although various studies have established the benefits of thermal gradients with open tubular capillary columns, little has been done using microchip columns under TGGC conditions. Our research has shown that TGGC is well suited for enhancing the performance of microchip gas chromatography columns where non-instantaneous sample introduction, multiple turns, various sources of dead volume, non-uniform stationary phase coatings, and presence of active sites make separations less than ideal. In this work, microchip GC columns were fabricated from silicon substrates, and then deactivated and coated. Simulations of heating along the microchip GC columns were correlated with various heating scenarios. Close correlation between simulated and measured temperatures allowed for detailed analysis of the temperature gradient along the column and correlation with its performance in chromatographic separations. Chromatograms were obtained under controlled heating conditions according to the thermal gradients optimized through thermal modeling.
Previously, we have shown the dramatic improvement of on-site analyses using portable equipment. This was accomplished by the incorporation of compounds referred to as “Focusing Agents” (FA) onto thermal desorption (TD) tubes. We further improved the technique by employing isotopically labelled analogs onto TD tubes, which was followed by collection and analysis. Data precision and accuracy allowed measurement of chemical warfare agents to 1 nanogram per tube when analyzed by a portable gas chromatographic mass spectrometer (GCMS). However, quantification using the hand probe accessory remained problematic for the introduction of semi-volatile calibration standards under field conditions or in the laboratory. Today we discuss a technique that yields improved precision and accuracy. We developed with industry, a hand-held device/“tool” that contains a MEMS chip that can load exact amounts of calibration standards from an internal reservoir. This is the “micro-calibrator.” In this manner the compounds can be loaded directly into the hand-held probe. This calibration tool is easily transportable and eliminates the needs of standard preparation under field conditions. In addition to the development of a calibration tool, the sampling profile required modification to introduce the FAs. This was done in conjunction with programming where the calibration compounds are transferred from the tool to the probe and then to the portable GCMS’ internal concentrator prior to sample collection. The samples are then collected onto the concentrator that now contains the FA standards. This in situ method greatly improves the data quality when compared to external standard methods. The Micro Calibrator using focusing agents provides a process which can be collected and analyzed under field conditions yielding highly reliable data.

Keywords: Gas Chromatography/Mass Spectrometry, Method Development, Portable Instruments, Sampling
Application Code: Homeland Security/Forensics
Methodology Code: Sampling and Sample Preparation
Hyperspectral imaging (HSI) sensors are a valuable tool for the detection of threat materials (explosives, narcotics) located within complex environments, where the concentration of the target of interest is low and the complexity of the background materials can often inhibit the detection of the threat material. In order to deploy a sensor capable of successful operation in these conditions, advancements to the current generation of HSI sensors must be achieved. This is because current generation HSI systems have size, weight, and power (SWaP) limitations that prohibit their use in these environments or they cannot adequately analyze an area of interest at a fast enough rate. In addition, many require close proximity to the target area, which increases the potential threat to the operator.

ChemImage Sensor Systems (CISS) has developed a standoff shortwave infrared (SWIR) hyperspectral imaging sensor called VeroVision, that combines an expanded area of interrogation with automated detection algorithms to provide the operator high confidence detection results in near real-time. VeroVision has been designed to detect homemade and military grade explosives and narcotics, using a simple to use software package that provides the user a the location of the threat material. VeroVision utilizes a multi-conjugate filter (MCF), which allows the sensor the ability to adapt to changing mission needs and expand its explosive and narcotic libraries as needed.

This presentation will provide background on hyperspectral imaging and SWIR spectroscopy, discuss system design, and present detection results for explosive and narcotic materials.

**Keywords:** Detection, Imaging, Instrumentation, Vibrational Spectroscopy
**Application Code:** Homeland Security/Forensics
**Methodology Code:** Sensors
Homeland Security/Forensics - On-Site Instrumentation

Development of Low-cost Portable Electrochemical Instrumentation for Automated On-site Analysis

Developments in microcontroller and battery technologies have facilitated the development of several advanced portable instruments. Over the past year, we have developed a portable electrochemical analysis platform robust enough for highly sensitive on-site measurements. This instrument is capable of autonomous on-the-fly gain control and self-calibration, and has a linear range spanning from 200 pA to 1 mA. It has a sweep rate range of 1 - 400 mV/s, and is currently programmed to perform several electrochemical experiments including cyclic voltammetry, anodic stripping voltammetry, linear sweep voltammetry, and potential step voltammetry. This instrument is capable of performing these electrochemical experiments autonomously, independent of user input. Further, we developed software to allow this instrument to analyze electrochemical data autonomously. These automated features afford unskilled users the ability to collect and analyze electrochemical data, without the need for extensive training. To increase this instrument's utility in the field, it was built into a water-proof hard case, and designed to be as inexpensive as possible. As a result, this instrument is both rugged and affordable, with material costs amounting to just a few hundred dollars. The instrument also features the ability to be powered by either an internal lithium polymer battery, or an external power source, allowing it to be used in the field for up to 15 days, or in the lab. This instrument was used to perform analysis of metals in lake water. Lead and copper were quantified on-site, with no peripheral equipment, with limits of detection in the single parts per billion range. In addition to environmental testing, this platform will be used in conjunction with electrochemical binding assays for analysis of biologically relevant analytes.

Keywords: Electrochemistry, Environmental/Water, Forensic Chemistry, Portable Instruments

Application Code: Homeland Security/Forensics

Methodology Code: Electrochemistry
Organizations sometimes opt out of purchasing a new or replacement LIMS solution for their laboratories because of the capital investment costs. While upfront cost is a significant consideration, companies need to weigh this against the costs of not being automated. The capital investment of a LIMS can be eclipsed by the fallout and costs from single outbreak or regulatory violation.

Without automation, laboratories may have limited throughput, increased costs for reporting, human errors, and inefficient processes. While these areas may have only a minimal impact on operations, other aspects have the potential to create significant and costly problems.

Staff salaries comprise a major portion of the laboratory’s budget, and many labs are attempting to do more with fewer team members. This puts organizations without automation, or with limited LIMS functionality, at risk for human error, limits throughput, and extends the typical turnaround time for completing sample analysis. Additionally it may lead to the most serious ‘cost’ of not being automated.

Compliance violation fees can run into the tens of thousands of dollars, which may completely overwhelm smaller labs, or substantively hamper operations in larger laboratories. If the cost of an automated sample data-management system seems scary, it is important to consider the potential damage of not having one.

This presentation will highlight specific areas that often cost laboratories without automation more in the long term, and briefly touch on how organizations will save money with automation.
The Water Pollution Control Laboratory at the City of Clearwater, FL provides the community of 108,000 with safe drinking water and supports a proactive wastewater collection and treatment system. The WPC Laboratory tests drinking water, wastewater and solid materials for three wastewater plants and the reverse osmosis plant, performing approximately 41,000 tests annually. In addition, the lab performs quality control and regulatory compliance analysis and monthly bacteriological analyses are run for the community drinking water system.

The lab previously conducted their testing using bench sheets and a very labor-intensive manual entry process that was prone to data transcription errors. Eventually the lab was able to justify the purchase of a LIMS and the impact and the benefits to the organization and their residents has been enormous.

This presentation will focus on the many benefits that have been realized due to the implementation of the LIMS including the following:

- A significant increase in productivity throughout the laboratory. In many cases, data is entered only once, eliminating transcription errors due to entering data in multiple documents.
- Significantly reduced turnaround times for data analysis and reporting – end result are happy clients who get their reports quickly.
- The LIMS generates the reporting required to meet federal and state regulatory compliance requirements for drinking water and wastewater monitoring.
- Clients can actually access their reports 24/7 via access to a secure web-based portal. This solution not only enhances customer service but also allows lab staff to focus on analysis and not having to respond to simple customer requests for information.
- Ability to schedule tasks increases the efficiency of the lab operation by eliminating the need to perform these repetitive tasks.
- Increasing demand for laboratory data by leadership from other city departments.

Abstract Text

The Water Pollution Control Laboratory at the City of Clearwater, FL provides the community of 108,000 with safe drinking water and supports a proactive wastewater collection and treatment system. The WPC Laboratory tests drinking water, wastewater and solid materials for three wastewater plants and the reverse osmosis plant, performing approximately 41,000 tests annually. In addition, the lab performs quality control and regulatory compliance analysis and monthly bacteriological analyses are run for the community drinking water system.

The lab previously conducted their testing using bench sheets and a very labor-intensive manual entry process that was prone to data transcription errors. Eventually the lab was able to justify the purchase of a LIMS and the impact and the benefits to the organization and their residents has been enormous.

This presentation will focus on the many benefits that have been realized due to the implementation of the LIMS including the following:

- A significant increase in productivity throughout the laboratory. In many cases, data is entered only once, eliminating transcription errors due to entering data in multiple documents.
- Significantly reduced turnaround times for data analysis and reporting – end result are happy clients who get their reports quickly.
- The LIMS generates the reporting required to meet federal and state regulatory compliance requirements for drinking water and wastewater monitoring.
- Clients can actually access their reports 24/7 via access to a secure web-based portal. This solution not only enhances customer service but also allows lab staff to focus on analysis and not having to respond to simple customer requests for information.
- Ability to schedule tasks increases the efficiency of the lab operation by eliminating the need to perform these repetitive tasks.
- Increasing demand for laboratory data by leadership from other city departments.
An automated laboratory strives to monitor processes and track inventory such as compound libraries and equipment. With governance and the push to get drugs on the market faster, IT infrastructure must tie the labs closer to their business. We are presenting novel and easy to use technologies that are scalable, flexible, profit enhancing, and cost effective approach to laboratory real time performance monitoring. We have been adopting use of integrated lab standards like AniML and CAN open-based device profiles. The system is queried with instrument usage, experimentation progress, lab performance, throughput, downtime, frequency of use, time to validate, reagent use etc. In addition, we will be discussing log gathering and analysis of laboratory systems. We will overview Laboratory Asset and Facility Management Systems on the market and provide you with the parameters and requirements needed in choosing a system and managing a system. Systems that will be demonstrated are free open source systems and well supported systems such as IBM Maximo. A review of user and industry required system features and components such as Calibration, Purchasing, Inventory, Contracts, Service, Preventative Maintenance, Repair, and other modules relevant to the laboratory business. We will demonstrate tools available for automated calibration, validation, work ordering, PM, service reporting, inventory, procurement, auto-discovery, asset tracking, data tracking, and remote control.

Keywords: Lab Management, Laboratory Informatics, LIMS, Software
Application Code: Laboratory Management
Methodology Code: Laboratory Informatics
Regardless of what type of laboratory is being evaluated, there are five (5) key trends that have been on the rise and known to be impacting laboratories in a negative way:

1) Increase in Transcription of Data
2) More Companies Are Managing Multiple Data Locations
3) Use and Management of Paper Processes Is Increasing
4) Resource Workload Higher Than Resource Capacity
5) Increased Quality Issues: Investigations and Corrective Actions and Preventive Actions (CAPA)

With the lack of automation or with partial automation of a laboratory, many of these trends will continue to operate and will become exponentially more challenging as companies attempt to scale.

All of the above trends are directly linked to each other. They happen for a number of reasons, improperly configured LIMS, lack of current LIMS or database functionality, outdated LIMS doesn’t meet current operational needs, temporary documentation during analysis due to lack of local LIMS access, etc. All of these trended risks cost time, lead to errors, overwhelm current resources and capacity, and involves management of multiple systems which leads to variance across systems and challenges data integrity.

To mitigate these trend risks to operations, companies should implement the right-fit Laboratory Information Management System (LIMS) and/or Electronic Laboratory Notebook (ELN) and interface the LIMS with instruments and other software to automate data movement and increase data integrity. By taking this approach, companies eliminate transcription of data, reduce the number of systems that data exists in, removes the need to manage paper processes, and reduces the number of quality issues associated to data entry and testing. Implementation and integration with a LIMS/ELN would generate higher resource capacity as their wasted time due to the other 4 trends would be reduced drastically, freeing them up to perform more value-added work.
Here we present a novel mode in atomic force microscopy (AFM), ringing mode, an extension of popular sub-resonance tapping modes (such as Digital Pulse, Peak Force Tapping, HybriD, etc.). This mode utilizes the signal information from free resonance oscillations of the cantilever, which occur after detaching the AFM probe from a sample surface (ringing). Processing the ringing signal allows recording simultaneously up to 8 new channels of information, such as adhesion height, adhesion neck height, detachment energy losses, size of possibly stretchable molecules, etc. In addition, Ringing mode can be up to 20 times faster and showing fewer artifacts compared to the existing sub-resonance tapping modes. We demonstrate the work of this new mode on complex samples, such as fixed human epithelial cells, corneocyte skin flakes, and polymeric nanocomposites. In addition, examples of unique information that can be seen only in ringing mode will be presented.

Abstract Text

Here we present a novel mode in atomic force microscopy (AFM), ringing mode, an extension of popular sub-resonance tapping modes (such as Digital Pulse, Peak Force Tapping, HybriD, etc.). This mode utilizes the signal information from free resonance oscillations of the cantilever, which occur after detaching the AFM probe from a sample surface (ringing). Processing the ringing signal allows recording simultaneously up to 8 new channels of information, such as adhesion height, adhesion neck height, detachment energy losses, size of possibly stretchable molecules, etc. In addition, Ringing mode can be up to 20 times faster and showing fewer artifacts compared to the existing sub-resonance tapping modes. We demonstrate the work of this new mode on complex samples, such as fixed human epithelial cells, corneocyte skin flakes, and polymeric nanocomposites. In addition, examples of unique information that can be seen only in ringing mode will be presented.

Keywords: Atomic Force Microscopy (AFM), Biological Samples, Polymers & Plastics, Surface Analysis

Application Code: Nanotechnology

Methodology Code: Microscopy
Nondestructive chemical, mechanical measurement of materials with sub 10 nm spatial resolution together with topography provide correlative information of on heterogeneous functional materials. However, such multimodal nanoscale chemical and mechanical correlations are difficult to achieve, due to the limitation on spatial resolutions of the optical microscopy and constraints from instrumental complexities. Here, we present a multimodal non-invasive spectroscopic scanning probe microscopy platform based on the peak force infrared (PFIR) microscopy. The method allows chemical sensitive imaging through mapping the infrared absorption through laser-induced thermal expansions, and simultaneous acquisition of modulus and adhesion through the peak force tapping. Both functionalities are obtained with sub 10 nm spatial resolution. PFIR is shown to be capable of measuring both soft materials and inorganic materials.

As a demonstration, the nanophase separation of a di-block copolymer of PS-b-PMMA was measured at characteristic infrared frequencies to reveal the distribution of constituent domains (see the attached figure). As high as 6 nm spatial resolution in the chemical sensitive imaging was demonstrated from the infrared imaging capability of PFIR the microscopy. This spatial resolution, in fact, surpasses many nanoscale infrared microscopy, such as the popular AFM-IR techniques. The detection limit of PFIR is demonstrated to be as low as 1100 vibrational modes or at the zeptomole-level. The high spatial resolution and high sensitivity enable the PFIR technique to be a powerful analytical tool for explorations at the nanoscale on heterogeneous materials.

Keywords: Atomic Force Microscopy (AFM), Infrared and Raman, Microscopy, Spectroscopy
Application Code: Polymers and Plastics
Methodology Code: Microscopy
A video rate hyperspectral two-photon fluorescence imaging system was demonstrated. This method acquired up to 18 frames per second with over 2000 effective spectral channels in a 200 nm wavelength window. With the high spectral resolution of this system, fluorophores with similar emission spectra, such as fluorescein and coumarin 6 can be classified with an iterative algorithm developed in house. Meanwhile, the high imaging speed of this system made it possible to study dynamic biological sample such as fluorescent tagged C. elegans. The ability to recover high resolution spectra provides additional layer of information comparing to conventional filter based fluorescence microscopes, which enables studies of various complex biological processes. Current hyperspectral imaging strategies can be generally classified as spectral scanning, spatial scanning, and snapshot methods. These systems typically have limited imaging speed due to the mechanical movement of wavelength dispersion optics, detector integration time, and/or require complex instrument setups. By utilizing the nature of beam scanning system in angle dependent signal collection, we reported a simple, fast two-photon fluorescence hyperspectral microscopy using a 16-channel photon multiplier tube (PMT). Instead of de-scanning optics, a 4f configuration was added in the collection path to lock fluorescence signals onto the center of a transmit diffraction grating, allowing PMT array to probe different sets of spectral windows at different pixels in the field of view. Since this setup required addition of a diffraction grating and a detector array to most commercial beam-scanning microscope, it is accessible in a wide number of research facilities.
The knowledge of rotational dynamics in and on live cells remains highly limited due to technical limitations. The differential interference contrast (DIC) microscopy-based Single Particle Orientation and Rotational Tracking (SPORT) techniques have been developed in the Fang Laboratory to acquire accurate measurements of anisotropic plasmonic gold nanorods in complex cellular environments. Rich information in five dimensions, including the x, y, z coordinates and the two orientation angles (azimuthal angle and polar angle) of the probe's transition dipole, can be obtained from SPORT experiments. The SPORT technique is capable of extracting important information (including rotational rates, modes, and directions) on the characteristic rotational dynamics involved in cellular processes, such as adhesion, endocytosis, and transport of functionalized nanoparticles, as may be relevant to drug delivery and viral entry.

Keywords: Imaging, Microscopy, Nanotechnology
Application Code: Bioanalytical
Methodology Code: Microscopy
Three-dimensional single particle tracking (3D SPT) is a powerful tool in various chemical and biological studies. In 3D SPT, non-conventional point spread functions (PSFs) are frequently used to generate different patterns, from which the axial position can be recovered in addition to the x-y coordinates. This requires complicated data analysis involving the recognition of irregular image patterns. In addition, noises and interfering background make the pattern recognition more challenging. Currently, the most used data analysis methods, i.e. correlation coefficient mapping-based methods, perform poorly in these situations. In this study, we developed a novel localization algorithm based on Deep-Learning Neural Networks. By incorporating artificial intelligence into the data analysis, we are able to improve the localization precision in the z-direction by 1 order of magnitude for low S/N ratio data. It allows us to improve the acquisition time to be as low as 50 microseconds for 200 nm fluorescent particles without losing precision significantly. Examples of particle diffusion in confined spaces and on small oil droplet surface are shown. This new method will greatly improve our capability in 3D single particle tracking.

Keywords: Bioanalytical, Imaging, Nanotechnology, Single Molecule
Application Code: Bioanalytical
Methodology Code: Microscopy
Suspension lipid bilayers, also called black lipid membranes (BLMs), are a common model of biological lipid membranes. BLMs are typically made by “painting” an organic solution of lipids over microfabricated apertures in an aqueous environment, resulting in self-assembled suspension lipid bilayers. BLMs offer an advantage over other membrane models, such as supported bilayers and liposomes, because they allow dynamic control of the solution environment at both faces of the membrane. Painting BLMs is a tedious manual process prone to operator-to-operator error and irreproducibility. Confirming the presence of manually painted BLMs often requires methods that are deleterious to the membrane or the experiment, such as insertion of pore proteins or electrical breakdown of the BLM. To address these limitations, we have developed an automated process for BLM formation involving precision control of applied pressure at the aqueous/organic/microaperture interface. Fluorescently labeled lipids constituted 1% (wt.) of total lipids in solution, and z-scanning confocal fluorescence microscopy is used to monitor BLM formation over time. As the applied pressure was increased, fluorescence signal decreased as the organic plug thinned to form a BLM. Simultaneous membrane pore conductance measurement and z-scanning confocal microscopy confirmed that the fluorescence signal can be used to verify the presence of a lipid bilayer. The automation of BLM formation and new methods of BLM characterization in microfluidic platforms will play important roles in the development of novel membrane-mimetic sensor systems.
Planar supported lipid bilayers (PSLB) composed of fluid lipids lack the stability necessary for many applications due to the relatively weak interactions between lipid molecules. Polymerization of PSLBs is one method to enhance bilayer stability, however it greatly reduces fluidity and influences mechanical properties. Experimental data demonstrate that some transmembrane proteins require a certain fluidity and elasticity of the membranes for their proper reconstitution and bioactivity. Thus, investigation of mechanical properties of PSLBs are essential in elucidating protein-based biosensor functions. Atomic force microscopy (AFM) force mapping directly correlates morphology with mechanical properties. Here we have prepared PSLBs composed of mixtures of bis-SorbPC (polymerizable lipid) and DPhPC (fluid lipid) to form fluid, yet stable bilayers. Nano-scale phase segregation of the two lipids was observed with AFM. Force mapping analysis of these PSLBs showed that the elastic modulus of poly(bis-SorbPC) domains were higher than that of DPhPC domains, suggesting that the polymer domain is stiffer. Breakthrough force for DPhPC was 3 nN(±1), whereas no breakthrough was observed for poly(bis-SorbPC) domains. This could also be attributed to higher stiffness of poly(bis-SorbPC), which prevented the AFM tip from penetrating the PSLB up to a force of 10 nN. Deformation of DPhPC was higher than that of poly(bis-SorbPC) and this difference agrees with the height difference that was observed with AFM topography. Furthermore, higher adhesion force revealed by DPhPC compared to poly(bis-SorbPC) domains could be due to higher tip-sample interaction in DPhPC caused by tip penetration. In summary, polymerized mixed PSLBs phase-segregate to form domains; where DPhPC domains allow protein insertion, while poly(bis-SorbPC) domains provide mechanical stability to lipid bilayers, thereby showing considerable potential as platforms for protein-based biosensors.

Funding: NIH R01EB007047

Keywords: Atomic Force Microscopy (AFM), Biosensors, Lipids, Membrane

Application Code: Bioanalytical

Methodology Code: Microscopy
Interaction of Picloram and Glyphosate with Giant Unilamellar Vesicles: Study of Their Disruption Properties on Artificial Membrane

We have assessed systematically the binding effect of picloram and glyphosate on an artificial membrane model: giant unilamellar vesicles (GUVs) constitute with sphingomyelin (SM), cholesterol (Chol), and the unsaturated lipid dioleoyl-sn-glycero-3-phosphocholine (DOPC) in a physiological environment. Simplified binary and ternary lipid model (DOPC/SM 1:1) and (DOPC/SM/Chol 1:1:1) membranes have therefore been reconstructed to gain insight into the synergetic role of lipids in pesticide-membrane interactions at the membrane surface. GUVs were prepared using both gel-assisted and electroformation techniques, and have been used to mimic ocular membrane line in the eyes. We first validate the model using confocal microscopy to analyze the general phase properties of the system under ranges of temperature and composition. The phase contrast microscopy coupled with FTIR was employed to assess the spectroscopic properties (lipid acyl group packing and spectral shape) and sucrose leakage upon pesticides binding. Shape-induced such as protrusion, contouring, and elongation was observed when the bilayer surface was exposed to pesticide concentrations from 10-5 to 10-4 mol/L, which was attributed to the binding effect of the pesticides. We further observed the glucose leakage effect on the GUV at high pesticide concentration (10-3 mol/L), which strongly suggested pesticide adsorption to the head group of the bilayer and, as a result, it increases its permeability (which indicated the disruptive nature of the pesticides). Furthermore, FTIR analysis indicated minor changes in both the acyl chains and the head group region of the bilayer, suggesting some level of chemical interaction between the bilayer and the pesticides. Eventually, our finding can provide a great resource for investigating the solubility of certain hazardous pesticides on the surface of the membrane, and offer a paradigm for understanding the dynamic nature of such model. These results and tendencies for further probe with selective outcome of successful applications of the techniques are the aims of this investigation.

Keywords: Fluorescence, FTIR, Lipids, Pesticides
Application Code: Bioanalytical
Methodology Code: Microscopy
The technique of infrared chemical imaging has been widely used for many industrial applications. It offers selectivity and/or sensitivity for numerous organic functional groups. The advantage of the near infrared region of the electromagnetic spectrum is the linear relationship of Absorbance and concentration that enables quantitation. This universally employed technique has been a boon for our research studies in the industrial process of wheat milling for the production of flour. This process has numerous sequential grinding and sieving steps that enable selective physical segregation of a starch rich endosperm product from wheat. Thousands of spectra of purified endosperm and non-endosperm standards are collected to develop a spectral library. Quantitation of the purity of individual processing streams is accomplished by applying a partial least squares calibration that is based upon the spectral library. Examples are presented of a new method for optimization of the milling process. This new method would not be possible without the sensitive and selective method of quantitative chemical imaging.

**Keywords:** Imaging, Method Development, Near Infrared, Optimization

**Application Code:** Process Analytical Chemistry

**Methodology Code:** Near Infrared
Hydrophobic interaction chromatography (HIC) is a non-denaturing separation technique to maintain protein structure and biological activity. However, HIC possesses several disadvantages including low resolution and binding capacity, dramatic baseline shifts during gradient elution, and a requirement for non-volatile mobile phases, which limits extended characterizations. In this regard, we developed polymer-bonded phase HIC column for the purpose of applying mass spectrometry (MS)-compatible salts and buffers using ammonium acetate to achieve higher resolution separation and minimize baseline changes on a biomimetic ADCs from DAR 0 to DAR 8. Furthermore, as the demand of online HIC-MS applications, the lower concentration (< 1.5 M) on ammonium acetate was achieved to perform good baseline separations on the ADCs.
Real time, point of sampling analysis detection and identification of trace materials (hazardous or benign) is of utmost importance amongst diverse fields viz. Department of Defense, homeland security, medical, environmental, industrial and forensic analysis. Herein, we design a novel and simple SERS substrate by impregnating various gold nanostructures (Au NS) onto a chitosan film and bringing the Au NS close to each other and creating electromagnetic hot spots. These hot spots allows sensitive detection of trace analyte molecule using SERS technique. The ease of fabrication of the SERS substrate and the ability of synthesizing nanostructures using a simple chemistry lab, and the availability of portable handheld Raman spectrometer will enable rapid point of sampling analysis of trace materials in the field of forensics, food safety, defense, standoff detection and environmental protection. In addition, this would help reduce the cost and time delays associated with a central lab model common to regulatory and accreditation agencies, as well as open new avenues for analysis of labile samples that would decompose or otherwise change between the point of sampling and the laboratory in which they are analysed.

Keywords: Analysis, Nanotechnology, Surface Enhanced Raman Spectroscopy, Trace Analysis
Application Code: Process Analytical Chemistry
Methodology Code: Sensors
Europium is one of the important lanthanide elements has applications in colour TV, street light, ion selective electrodes, magnetic materials, etc. In nuclear industries europium has an important role in controlling excess reactivity for thermal reactors. Polymeric membrane-based sensors containing N,N,N',N'-tetraoctyl diglycolamide (TODGA) or N-pivot diglycolamide extractant (DGA-TREN),1 as the ionophore and polyvinyl chloride (PVC) and cellulose triacetate (CTA) as the polymer were fabricated. The TODGA-based membrane, constituted with a composition of 30.3% TODGA, 9.1% NaTPB and 60.6% PVC, showed a detection limit of 1.2x10^-6 M, whereas the DGA-TREN containing membrane had a detection limit of 3.9x10^-7 M. The detection limits of the DGA-TREN-containing membrane were found to be marginally better compared to those obtained with the TODGA-containing membrane. This has been ascertained from AFM and electrochemical impedance measurements. Some AFM scans and calibration plots of the membranes are shown in Figure 1. The AFM scans show no significant variation of the morphology due to swelling on overnight treatment in an Eu^{3+} ion containing solution. Electrochemical impedance measurements revealed an increase in conductivity of the films on prolonged use, indicating inclusion of ions inside the films.
### Sensors - Bioanalytical

**Quantitative Detection of Targets in Single Cells with Plasmonic Assemblies**

Discrete plasmonic multi-nanoparticle assemblies are invaluable for studying biomolecular interaction and for chip-based sensing. Although plasmonic assemblies are widely explored for sensing single-molecule binding events, the quantitation of target of interest is challenging due to the narrow dynamic range. Additionally, single plasmonic nanostructure spectroscopy requires sensitive detectors and spectrographs that are not readily available to researchers outside the plasmonics field. Herein, we demonstrate an approach through nanoparticle-assembly design on a chip to amplify the optical detection signal that may facilitate practical integration of these sensors with portable and consumer-grade instruments. We demonstrate the layer-by-layer assembly of satellite nanoparticles around core nanoparticles; the iterative layer buildup process can theoretically yield limitless enhancement in plasmon coupling and scattering intensity. Detection via the disassembly of the structures yields a dramatic decrease in scattering intensity that can be quantitated through simple darkfield image analysis. We show that the sensing performance, such as detection limit, sensitivity and dynamic range, can be tuned by controlling the assembly size. The nanoparticles linked by the structural-switching aptamer can directly detect the exemplary target (ATP) from lysed cells without any purification or separation. Finally, we develop a method to quantitate the ATP levels in individual cells by mapping the microenvironments near the nanoparticle assemblies and discuss the potential for multiplexed detection of different biomolecules. The methodology can be readily adapted for different targets and may be developed as a low-cost and rapid bioanalytical platform for single-cell analysis. Our work addresses the challenges of chip-based nanoparticle sensing platform and may pave the way for their use in broader applications.

This work is supported by funds from NSERC of Canada and CIHR.

**Keywords:** Bioanalytical, Biosensors, Microscopy, Nanotechnology

**Application Code:** Bioanalytical

**Methodology Code:** Sensors
Increased Volume Responsiveness of Macroporous Hydrogels

Hydrogels can be fabricated into smart materials whose volumes predictably depend on their chemical environment. These smart hydrogel materials can be utilized in applications such as sensors, actuators, and for drug delivery materials, for example. The volume response of these hydrogels is well-known to be limited by their crosslink density. Thus, the responsiveness of hydrogels can be increased by decreasing the hydrogel’s crosslink density. Unfortunately, this also decreases the hydrogel strength.

The hydrogel “effective crosslink density” can be decreased by fabricating macroporous hydrogels where voids are incorporated into the hydrogel. In the work here we demonstrate that this approach increases the volume responsiveness of hydrogels. We fabricated pH responsive macroporous hydrogels by copolymerizing acrylic acid with acrylamide. We compared the pH response of these hydrogels to that of macroporous hydrogels with small water bubbles embedded by vortexing the polymerizing hydrogel in air, or by preparing an inverse opal hydrogel. We then filled these embedded air bubbles with water. The pH responsiveness of these macroporous hydrogels are significantly increased compared to those of non-macroporous hydrogels of similar composition. We find that these macroporous hydrogels appear to be more mechanically robust than are similarly responsive hydrogels without voids.

Keywords: Polymers & Plastics, Sensors
Application Code: Polymers and Plastics
Methodology Code: Sensors
There is a need to develop point-of-care blood phenylalanine (Phe) detection instruments analogous to home glucose meters for phenylketonuria patients who need regular monitoring of Phe concentrations. We developed 2D photonic crystals (2DPC) responsive hydrogel materials for the detection of phenylpyruvate (PhPY), an enzymatic by-product of Phe and phenylalanine dehydrogenase (PheDH). The PhPY detecting hydrogels were fabricated by copolymerization of monomers including tert-butyl(2-acrylamidoethoxy)carbamate (TBAC). TBAC is a newly synthesized monomer which has an oxyamine recognition group that can react with PhPY. The reaction between PhPY and the recognition groups induce osmotic pressures in the hydrogels that swell the hydrogel volume. This PhPY induced swelling increases the particle spacing of 2DPC embedded in the hydrogels. This result is in angle-shifts of the diffracted light that can be used to monitor the hydrogel volume. The attachment of PhPY to the hydrogel and the chemistry of the hydrogel volume changes were studied by NMR spectroscopy and 2DPC light diffraction measurements. These 2DPC hydrogels showed linear volume responses to PhPY concentrations. The hydrogel volume increased up to 37 % in response to 5 mM concentration PhPY. The calculated limit of detection for PhPY was 1.43 mM and the detection time was less than 1.5 hours. This short PhPY detection time is a significant improvement over existing clinical methods that take days to determine blood Phe concentrations. The PhPY detecting hydrogels are being developed to measure enzymatically produced PhPY from Phe and PheDH for the development of point of care Phe sensors.

Keywords: Bioanalytical, Biosensors, Sensors
Application Code: Bioanalytical
Methodology Code: Sensors
Electrophoretic separations conventionally rely upon chromogenic, fluorogenic, or redox active characteristics for analyte identification. Universal detectors are an attractive alternative to these labelled methods because they enable detection of analytes without these chemical signatures and can be performed without analyte modification (e.g., adding fluorescent tags). In this work, we demonstrate silicon photonic microring resonator arrays as a platform for detecting bulk changes in local refractive index (RI) interfaced with electrophoresis. Here, an applied electric field drives charge-dependent migration of different chemical species across a novel 3D-printed device. The migration of these molecules is tracked in real-time by leveraging the efficient readout of spatially-defined sensors. The ability to manipulate charged species is a promising approach for complex sample handling and label-free analysis. Bulk RI detection is less sensitive than UV/Vis or fluorescent methods, and for applications where the RI detection limits are insufficient, targeted capture agents can be arrayed on the across the microchip surface to leverage the exquisite surface sensitivity of the silicon photonic platform. These sensors have been widely implemented as multiplexed biosensors using pressure-driven flow applications, but this is the first demonstration of a silicon photonic sensor array with electrophoretic driven flow. To that end, we demonstrate proof of concept experiments detecting DNA sequences driven across the sensor array via electrokinetic migration. We further show electrophoresis as a means of faster analysis with reduced input. Electrophoresis is appealing for silicon photonic sensors because of its ability to pre-concentrate samples, more efficient mass transfer compared to laminar flow, and the improved fluidics compared to traditional pressure driven-flow.

Keywords: Bioanalytical, Biosensors, Electrophoresis, Lab-on-a-Chip/Microfluidics
Application Code: Bioanalytical
Methodology Code: Sensors
Simultaneously knowing the expression level and location of target nucleic acid sequences will give insights over their role in regulating biological functions. In plants, this knowledge can be exploited for bioenergy purposes, as microRNAs have been shown to regulate key pathways for plant biomass accumulation. However, traditional approaches for RNA analysis (e.g., Northern blot, PCR) cannot allow monitoring these targets in live plants with the required spatial and temporal resolution. Biosensors based on surface enhanced Raman scattering (SERS) can be used to monitor/image nucleic acid expression in vivo and offer several advantages over other sensing platforms, including high sensitivity and multiplexing. Our group has developed a sensing system based on SERS that allows for the label-free detection of nucleic acids, the inverse molecular sentinels (iMS). These biosensors are composed of a nanostar (the plasmonic element necessary for SERS) coated with a target-responsive double-stranded DNA sequence bound to a Raman reporter. In the presence of the target sequence, one of the strands is removed from the sensor and the Raman reporter is brought in proximity to the nanostar surface via the self-hybridization of the remaining strand, generating a large increase in SERS signal. This mechanism allows for an off-to-on detection of target nucleic acids without the need of directly labeling the target. This paper will discuss the use of iMS for the detection of nucleic acid sequences relevant to bioenergy. Additionally, the recent development of an imaging system to monitor target nucleic acids in plants will be presented.
MicroRNAs (miRs) are small non-coding RNAs that regulate gene expression. The expression of specific miR combinations are associated with various biological processes from homeostasis to disease. Analytical tools, such as nucleic-acid-based logic sensors, are gaining popularity to provide \textit{in situ} analysis of specific miR combinations. Many published logic sensors suffer from poor sensing accuracy and a limited number of inputs. Such logic sensors impede reliable results and obscure the miRs’ biological roles. The sensor field needs to improve the accuracy of miR combinations because different numbers and combinations of miRs indicate different biological events. Here we will present research on the essential bio-recognition properties of a three-miR-input logic sensor referred as an “AND conveyor belt”. As a key part of the AND conveyor belt, a single-stranded-DNA known as a ‘probe’ is engineered to accommodate one blocking strand, two reporter strands, and three analyte-miRs through partial-complementary base pairing. Two reporters, one labelled with a donor dye and the other with an acceptor dye, induce Förster Resonance Energy Transfer (FRET) to generate the sensor’s ON state. Three analyte-miRs trigger a series of toehold-initiated strand displacement reactions that relocate the two reporters on the probe and bring them together to turn the signal “ON”. In order to improve the sensing accuracy, the AND conveyor belt introduces a blocking strand to hide the binding sites (toeholds) for the analyte-miRs and the reporters’ ON state. Such a design also holds the potential to scale up to as many as 10 miRs. We will discuss the AND conveyor belt’s detection principle, sensing accuracy, selectivity, and sensitivity.
To address the intriguing challenge of the direct detection of whole-cell bacteria in health care applications and the prevention of nosocomial infections, a localized surface plasmon resonance (LSPR) aptasensor was developed, in which \textit{Pseudomonas aeruginosa} strain PAO1 was used as a model organism and a \textit{P. aeruginosa} specific aptamer was used as an affinity reagent. The sensor surface containing a hexagonal array of gold nanotriangles was fabricated using nanosphere lithography (NSL), and was subsequently modified with biotinylated polyethylene glycol (Bt-PEG) thiol/PEG thiol (1:3), neutravidin, and biotinylated aptamer in a sandwich format. To maximize PAO1 binding while minimize nonspecific adsorption and steric hindrance, the Bt-PEG thiol/PEG thiol ratio and post-incubation washing steps were optimized. In contrast to prior whole-cell LSPR work, the LSPR wavelength shift was shown to be linearly related to bacterial load in solution over a range of bacterial loading 10 - 10^{3} \text{ cfu mL}^{-1}. This LSPR sensing platform is rapid (~ 3 h for detection), sensitive (down to a single bacterium level), selective for the detection of \textit{Pseudomonas} strain PAO1 over other strains, and it exhibits a clinically relevant dynamic range and an excellent shelf-life (\textasciitilde 2 months) when stored at ambient conditions. This versatile LSPR sensing platform should be extendable to a wide range of supramolecular analytes, including both bacteria and viruses, by simply switching affinity reagent, and it has the potential to be used in point-of-care and field-based applications.
Sensors - Bioanalytical

Probing the Dynamic Interaction between Damaged DNA and a Cellular Responsive Protein Using a Piezoelectric Mass Biosensor

The binding events between damaged DNA and recognition biomolecules are of great interest for understanding the activity of DNA-damaging drugs and the related DNA repair networks. Herein, a simple and sensitive sensor system was tailored for real-time probing of the dynamic molecular recognition event between cisplatin-damaged-DNA (cisPt-DNA) and a cellular responsive protein, highmobility-group box 1 (HMGB1). By integration of flow injection analysis (FIA) with quartz crystal microbalance (QCM), the interaction time-course of cisPt-DNA and HMGB1 domain A (HMGB1a) was investigated. The highly specific sensing interface was carefully designed and fabricated using cisPt-DNA as recognition element. A hybrid self-assembled monolayer consisting of cysteamine and mercaptohexanol was introduced to resist nonspecific adsorption. A linear range of 0.2-2 \text{M} was obtain for the detection of HMGB1a. And the limit of detection was estimated to be 60 nM (S/N=3). The calculated kinetic parameters (k_{ass} and k_{diss}) and the dissociation constant (K_D) demonstrated the rapid recognition and tight binding of HMGB1a toward cisPt-DNA. Molecular docking was employed to simulate the complex formed by cisPt-DNA and HMGB1a. The result indicated the cooperation effect of stacking and hydrogen bonding reinforces the interaction and stabilizes the complex. This continuous-flow QCM biosensor is an ideal tool for studying specific interactions between drug-damaged-DNAs and their recognition proteins in a physiological relevant environment, and will provide a potential sensor platform for rapid screening and evaluating metal anticancer drugs.

Reference:

Keywords: Bioanalytical, Biosensors, Nucleic Acids, Protein
Application Code: Bioanalytical
Methodology Code: Sensors
Aggregation of some proteins are implicated in serious human diseases, e.g. Amyloid [beta] in Alzheimer’s Disease (AD) or Amylin in type II diabetes. However, a fundamental understanding of the initial step of toxicity is still lacking, though membrane interaction is believed to be a key. A vexing question in the field is that we do not know how the toxicity of the small oligomer (n-mer, with n<20) varies with n. Here we have asked the question in the context of the interaction of Amylin oligomers with lipid bilayers using Single Molecule Photo-bleaching Microscopy technique. We probed Amylin monomers and oligomers first in the solution phase which yields concentration dependant monomer-oligomer equilibrium. We also expose these oligomers to supported lipid bilayers and measured their stoichiometry on the membrane. The comparison of solution state and membrane phase stoichiometry gives us the relative affinity of oligomers with different ‘n’. Our finding suggests that solution state aggregates are dominated by monomers and dimers. However membrane structures shows very few monomers and are dominated by dimers and trimers. Hence, we have been able to determine the equilibrium thermodynamics of the initial steps of aggregation and membrane attachment.
Very recently, Miami has emerged as the US epicenter of the now-global Zika virus (ZIKV) epidemic and there are no commercially available point-of-care tests available to detect active ZIKV infection. About 10% of pregnant women in the US with confirmed Zika infection had a fetus or baby with birth defects in 2016 according to the Center of Disease Control. Therefore, there is an urgent need to identify individuals at risk for ZIKV infection, especially pregnant women. To that end, we developed a highly specific sandwich-format colorimetric immunoassay for the detection of virus in the acute phase. The specificity of the assay is due to the use of a highly specific anti-Zika antibody that we isolated from humans and cloned. This antibody recognizes only ZIKV and no other Flaviviruses that typically present cross-reactivity with ZIKV. Optimization of the assay was performed employing the checkboard method to determine the optimal reagent concentrations, incubation times for each step of the immunoassay, reproducibility of the assay on separate days. The coefficients of variation were calculated for 3 separate concentrations that span over the linear range of the assay. Our immunoassay is selective, precise, and has a linear range of detection spanning over 3 orders of magnitude. We then performed validation experiments, including spike recovery studies and limit of quantification, for presence of ZIKV particles in physiological fluids such as urine, serum, saliva, and whole blood. Our assay sensitivity is similar in both buffer and neat urine, suggesting that urine has no or negligible matrix effects and is a good physiological fluid for the detection of acute phase ZIKV infection. The detection limits fall within the range of reported ZIKV levels in urine. Finally, we adapted the immunoassay developed into a point-of-care device that is based on lateral flow in a paper substrate that accurately detects the presence of ZIKV in urine in spiked samples.

Keywords: Bioanalytical, Biotechnology, Immunoassay, UV-VIS Absorbance/Luminescence
Application Code: Bioanalytical
Methodology Code: UV/VIS
Abstract Text
Proteins and other biomedia are prone to adsorbing or fouling on stainless steel surfaces, leading to inaccuracies in clinical assays and detrimental downtime of the analytical system. This paper will introduce a silicon-oxygen-carbon (carboxysilicon) coating that provides biochemical inertness, corrosion resistance, and low energy properties to stainless steel and other substrates. By using a vacuum thermal process instead of dip, spray, or line-of-sight deposition methods, the thermal chemical vapor-deposited (CVD) coating lends itself to ease of processing, high volume scale-up, and uniform deposition onto complex geometry components with narrow internal cavities, high aspect ratio features, and blind holes. Unlike solvent-based or crystalline deposition treatments, this amorphous, molecularly-bonded CVD coating can be flexed without risk of flaking or delamination.

After an introduction to the composition and deposition process, the protein-resistant, corrosion-resistant, and hydrophobic properties of this coating will be demonstrated and discussed. The CVD coating will also be compared to a fluoropolymer coating in the context of a clinical diagnostic application.

Keywords: Bioanalytical, Biomedical, Paint/Coatings, Protein
Application Code: Bioanalytical
Methodology Code: Process Analytical Techniques
Orientation analysis of collagen by second harmonic generation (SHG) is demonstrated using an approach that is immune to artifacts from birefringence and partial/total depolarization of the incident light. As a second order nonlinear optical process, SHG is a great method for tissue analysis due to its deep penetration capability. The symmetry requirements of SHG make it sensitive to polarization dependent measurement, providing rich information about local structures. Polarization dependent SHG has been a powerful tool for structural characterizations of myosin, collagen, microtubule organizations, pharmaceutical crystals and other chemistry or biological samples. However, as the penetration depth improved, partial or complete depolarization of the incident light complicates the analysis of polarization dependent SHG measurement. In this work, we embrace depolarization. A new mathematical framework was developed describing the polarization dependent SHG from a depolarized incident light. By bridging Jones and Mueller frames, the SHG could be connected back to a well-established and simpler Jones architecture. The prediction of the model agreed greatly with the experimental observation for z-cut quartz as a model system using both pure polarized and unpolarized incident light. Based on this framework, polar and azimuthal orientation for collagen fibrils were recovered on a per-pixel basis from the polarization dependent SHG generated from a depolarized source. This framework allows the potential application of partial depolarized incident beam in polarization dependent measurement for deep tissue imaging and structure analysis.

Keywords: Bioanalytical, Biological Samples, Imaging, Microscopy
Application Code: Bioanalytical
Methodology Code: Microscopy
Transport process across epithelium takes place by either transcellular pathways or paracellular pathways. At cell-cell junctions, paracellular pathways consist of tight junctions (TJs) regulated by TJ proteins. Since TJs limit the uptake and bioavailability of hydrophilic drugs, studying absorption enhancers which increase the permeability of TJs is important to drug delivery strategies. Among these enhancer reagents, sodium caprate has proven to reversibly open paracellular spaces by modulating TJ protein claudin-5 and tricellulin, but the defined mechanism is still unclear. To measure the effect of sodium caprate on epithelial transport, techniques using Ussing chamber and two-path impedance spectroscopy can be utilized to monitor paracellular conductance/resistance. While informative, they cannot collect characteristic responses from a single cell, or investigate the permeability change at tricellular TJs, where tricellulin is predominantly located. Recently, our group has developed potentiometric scanning ion conductance microscopy (P-SICM) which utilizes a double-barrel nanopipette and the ability of scanning ion conductance microscopy (SICM) to precisely control the position of the pipette over cell monolayer and measure the local potential generated by cell features, from which local conductance information at subcellular resolution can be calculated. Our previous work combined P-SICM with hopping mode techniques to allow nanoscale conductance mapping, which makes visualization of ion transport through subcellular structures possible. In this study, we use P-SICM to study transport properties of single cells under the influence of sodium caprate, with paracellular conductance and even tricellular conductance resolved. Time/Dose-response curves and recovery processes after removal of caprate are recorded. This study will potentially provide more information on the way sodium caprate alters paracellular pathways and insights for daily dose of caprate drugs.

Keywords: Bioanalytical, Microscopy, Nanotechnology
Application Code: Bioanalytical
Methodology Code: Microscopy
We present the development of a fiber-based platform for micro-3D printing and delivery of bacterial microclusters to remote environments. In these studies, protein-based microstructures are fabricated on the tip of a fiber using dynamic mask-based multiphoton lithography. Structures are fabricated around bacteria of interest which are allowed to proliferate to a desired phenotypic state, thus generating clinically relevant high-density but low-cell-count bacterial colonies, similar to those that seed nosocomial infections. The porous nature of these structures separate bacteria from their surroundings while still allowing for the exchange of nutrients, waste products, and other small molecules, such as those used for signaling. By fabricating structures on the tip of a micropositioned fiber, we are able to precisely tune where these microclusters are located in relation to varying stimuli, such as signaling molecules and nutrient sources, as well as other bacterial colonies. Specimens are imaged using different microscopies to monitor variations in bacterial population, including changes in growth rate, density of quorum sensing molecules used for cellular communication, and antibiotic resistance. Here, we report studies focused on the opportunistic pathogen *Pseudomonas aeruginosa*. Results from studies examining distance-dependent interactions between microbial clusters of varying size will be presented. Through this research, we will begin to better understand intricacies of sociomicrobial behavior and interactions, thus allowing for the creation of new ways to treat infections.

**Keywords:** Bioanalytical, Fiber Optics, Laser

**Application Code:** Bioanalytical

**Methodology Code:** New Method
We present the application of micro-3D (µ3D) printing techniques in combination with desorption electrospray ionization mass spectrometry (DESI-MS) to study sociomicrobial behavior in small (<10[^5] cells) dense (>10[^8] cells mL[^−1]) populations of bacteria. This allows us to track the production of important bacterial signaling molecules such as HSL and PQS. To accomplish this, multiphoton lithography is used to trap individual bacterial cells in biocompatible microscale structures fabricated from highly concentrated aqueous protein solutions containing photosensitizer. This µ3D printing technique provides precise control of bacterial spatial organization, creating aggregates of clinically relevant size and density. DESI-MS is used to subsequently analyze production of extracellular signaling molecules, metabolites, and membrane components to yield important information regarding these cells and their extracellular chemical environment. Combining the power of these two techniques with brightfield and confocal microscopy enables studies of complex communication mechanisms and spatial organization within monoclonal bacterial populations of interest. This research focuses on analyzing microstructures containing the opportunistic pathogen [i]Pseudomonas aeruginosa[/i], which is involved in a broad range of hospital acquired infections and secondary complications in cystic fibrosis patients. We present here studies of [i]P. aeruginosa[/i] temporal changes during growth and onset of pathogenic group behaviors, including development of biofilms and antibiotic resistance, using DESI-MS and microscopy on µ3D printed populations of varying size and shape. Comparing mass spectra of these populations at different time points to observed colony morphologies, cell counts, and cell densities can provide a better understanding of how infections develop, persist, and escalate in severity.

Keywords: Bioanalytical, Biospectroscopy, Electrospray, Mass Spectrometry
Application Code: Bioanalytical
Methodology Code: Chemical Methods
Bioanalytical and Neurochemistry

Lignin Degrading Enzymes Production by Fungal Cultures and Their Ability to Decolorize Selected Dyes

The production of the lignin degrading enzymes namely Laccase, Manganese Peroxidase (MnP) and Lignin Peroxidase (LiP) of selected white-rot fungus were examined in this study. After screening the cultures for the presence of the enzymes Laccase by Plate Assay techniques, quantitative analysis of all the three enzymes was done by Spectrophotometric method. Production was studied using liquid media. The effect of pH & different carbon sources namely Sugarcane bagasse, Rice bran and Wheat bran was examined on the production of these enzymes in liquid media. Effect of 2, 5-Xyldine on Laccase production was also assessed. As it is these enzymes that help in decolourization / degradation of dyes, the crude enzyme solution was also investigated for its ability to decolorize selected dyes.

All the cultures showed diverse enzyme production of all the three enzymes in different media & pH. All the selected dyes were decolorized by the crude enzyme extract in varying degrees. This study demonstrated that media manipulation can change the enzyme production in the fungal cultures. By optimizing the media condition we can maximize the desired enzyme production. The study also shows that rather than using fungi, is possible to directly use crude enzymes for decolourization of dyes & probably treat industrial effluents.

Keywords: Chemical, Environmental/Biological Samples, Environmental/Water, Enzyme Assays

Application Code: Bioanalytical

Methodology Code: Chemical Methods
Due to the extended half-life period, the clenbuterol (CLB) remained as a β-adrenergic stimulating drug for longer duration in the meat and liver of animals. Dual functionalized gold nanoparticles (AuNPs) were developed and utilized in colorimetric naked eye assay of CLB. The simple reduction method has been employed to dual functionalize the AuNPs by glutamic acid (Glu) and polyethylenimine (PE). The above AuNPs based conjugates of PE-Glu-AuNPs were found to be extremely stable at room temperature up to six months. Remarkably, the colorimetric assay of CLB by PE-Glu-AuNPs has been initiated at 300 nM at pH 5 and visualized through naked eyes visualized. Moreover, the UV-vis titration based sub nanomolar CLB detection has been estimated from standard deviation and linear fittings. The aggregation induced mechanism on CLB detection was well verified through Transmission electron microscopy (TEM) and dynamic light scattering (DLS) studies. The TEM studies supports the above aggregation induced CLB detection. The PE-Glu-AuNPs based CLB detection was not affected any other interferences. The sensor selectivity of PE-Glu-AuNPs to clenbuterol was also well demonstrated in human urine sample studies. PE-Glu-AuNPs with 0.7 μM CLB in advance demonstrated the effective CLB detection with 34 pM and 79 pM detection limits in deionized water and human urine, respectively.
Alpha-synuclein (aSyn) is a membrane-binding protein that is intrinsically disordered in solution, but is known to form an alpha-helical structure upon binding membranes. Due to its implications in Parkinson’s disease, aSyn has been a topic of research in order to better understand not only its role in the disease state, but also its physiological role in the cell. Through this work it has been shown that the membrane binding of aSyn is sensitive to both membrane curvature and charge as well as to the N-terminal acetylation of aSyn, a common post-translational modification. We are interested in further probing, in a site-specific manner, how the membrane binding of aSyn is altered by membrane size and charge as well as the effect that N-terminal acetylation of the protein has on membrane binding using electron paramagnetic resonance (EPR). In this work, aSyn was transformed and expressed in E. coli cells in the absence and presence of the fission yeast NatB complex, which enables the N-terminal acetylation of aSyn. Several cysteine mutants throughout the membrane binding region (residues 1-95) were generated and spin labeled. Spin labeled aSyn was then added to POPC/POPG lipid vesicles of varying curvature and charge, by altering the vesicle size and concentration of POPG, respectively. Through the use of continuous wave and power saturation EPR we can site-specifically measure the change in dynamics and membrane accessibility of aSyn as a function of membrane character and N-terminal acetylation to help elucidate the membrane binding behavior of this protein. This work is supported by Westminster College.
Silicon nanoparticles have been attracting a lot of interest in the field of biomedical applications due to their biocompatibility in vivo, as well as their flexible surface chemistry. Since a core region of the crystalline Si NPs is mostly protected from paramagnetic centers on its surface defect sites, the Si NPs conventionally represent extremely long depolarization times resulting in high MR signal-to-noise ratio with minimum background signals. In order to synthesize size-controlled Si NPs, we utilized magnesiothermic reduction of silica nanoparticles. Based on the traditional synthetic approach, we also produce $^{29}\text{Si}$ enriched NPs from synthesized $^{29}\text{Si}$-TEOS for the purpose of additional signal enhancements. The enriched Si NPs in combination with hyperpolarization technique provide sufficient MR signals for [i]in vitro[/i] and [i]in vivo[/i] MR applications. In conclusion, it suggests that the Si NPs can potentially be used as a biocompatible, targetable, and hyperpolarized MR Imaging probes.
N\textsuperscript{6}-methyladenosine (m\textsuperscript{6}A) is the most abundant and reversible post-transcriptional modification in eukaryotic messenger RNA (mRNA) and long non-coding RNA (lncRNA). The central role of m\textsuperscript{6}A in various physiological processes has generated a considerable biological and pharmacological interest. Alkbh5 (alkB homologue 5) belongs to the alkB family and is a non-heme Fe(II)/\textsuperscript{[alpha]}KG-dependent dioxygenase that selectively catalyzes the oxidative demethylation of m\textsuperscript{6}A. Herein, we report the backbone \textsuperscript{1}H, \textsuperscript{15}N, \textsuperscript{13}C chemical shift assignment of the 26-kDa human Alkbh5 protein. Assignments were acquired at 25°C by heteronuclear multidimensional NMR spectroscopy. In total, 90% of all backbone resonances were assigned, with 192 out of a possible 213 residues assigned in the \textsuperscript{1}H-\textsuperscript{15}N TROSY spectrum. The secondary structure, predicted by the program TALOS+ from the assigned backbone resonance, and the backbone amide Residual Dipolar Couplings (RDCs) measured on aligned samples of Alkbh5 are in good agreement with the previously reported X-ray crystal structure of the protein. Relaxation dispersion NMR experiments reveal conformational dynamics on the micro- to millisecond time scale throughout the entirety of the protein, suggesting that modulation of protein conformational dynamics by substrate/cofactor binding might be an important source of regulation for Alkbh5 enzymatic activity.

**Keywords:** Characterization, Environmental/Biological Samples, NMR, Protein

**Application Code:** Bioanalytical

**Methodology Code:** Magnetic Resonance
The interaction of cationic gemini surfactant hexanediyl-\(\text{-bis-}\)N-(2-hydroxyethyl)–N-methylhexadecylammonium bromide) and its monomeric counterpart N-(2-hydroxyethyl)-N,N-dimethylhexadecylammonium bromide on the conformational stability and antibacterial activity of hen egg white lysozyme was studied by using UV-visible, circular dichroism, intrinsic and extrinsic fluorescence, time-resolve fluorescence spectroscopy and docking method. Near and far-UV CD results suggested that cationic gemini surfactants have more pronounced stabilizing effect on the conformation of lysozyme as compared to its single chain counterpart which perturbs the secondary and tertiary structure of protein at higher concentration. Furthermore micelles of both the surfactants were observed to stabilize the conformation of the protein however gemini was found to stabilize it at much higher micellar concentration range. These results were further confirmed by other spectroscopic and molecular docking technique. In addition, cationic gemini surfactant was found to increase the antibacterial activity of hen egg white lysozyme imparting the usage of this study in pharmaceutical industries.
Protein is an expanding class of biotherapeutics with high potency to manipulate cell function and genetic information of cells for disease treatment. The low stability and cell penetration capability of proteins, however, challenges an efficient and effective protein therapy. In addition, new protein therapeutics that could be regulated and modulated by intracellular environment would allow the development of precision medicine for targeted disease treatment. In this presentation, I will be talking about my research in the past few years in the field of integrating synthetic protein chemistry with combinatorial lipid-based nanocarriers for developing new generation of protein-based nanomedicine. The chemically-engineered proteins have shown high potency for targeted cancer therapy by making use of intracellular environment to modulate protein function, as well as providing an effective approach to edit the genetic information of mammalian cells for correcting and treating genetic disordered diseases.

Keywords: Biomedical, Biopharmaceutical, Nanotechnology
Application Code: Biomedical
Methodology Code: Chemical Methods
The ability for bacteria to colonize a wound site and form biofilms is a leading cause of clinical infections within the US and abroad. These infections are responsible for millions of deaths every year and are often associated with implantable devices. In order to better understand the relationship between biomaterials and bacteria and prevent these infections, it is essential to probe the system at different stages within the biofilm life cycle. My research aims to understand various methods for killing bacteria in solution and in biofilm form as well as identify and evaluate novel platforms to be used as antibacterial surfaces. The use of varying assays to explore these complex systems has also brought forth a study whereby biological small molecules are tested against common cytotoxicity in vitro assays to show where interferences exist when using these assays. This involves a rigorous analysis of fifteen compounds tested against the MTT and resazurin-resorufin complex assays in the absence of cellular activity to identify where deviations from the control are present.
### Abstract Text

The multi-component reaction is a simplest and most reasonable methods for the development of biologically and pharmacologically active heterocyclic scaffolds. We synthesis it from the various heterocyclic aldehydes, ammonium acetate and different ketoester at ambient temperature using different catalyst. We found some of the catalyst is found be a very efficient for the multicomponent reaction. The obtained products were confirmed by the 1H-NMR, 13C-APT, IR and Mass spectroscopy. All compounds were screened for their microbial studies and some of the single crystals studies were reported.

### Keywords:
- Biomedical
- Characterization
- FTIR
- X-ray Diffraction

### Application Code:
- Biomedical

### Methodology Code:
- Chemical Methods
The aim of this study was to discover the role of leptin, adiponectin and resistin as a link between obesity and insulin resistance type 2 diabetes through assessment of their levels in normal weight, obese and obese diabetic subjects. Forty five subjects divided into four groups, 15 with normal weight as control group (group I), 15 obese subjects (group II), ten subjects from group II followed weight reduction for 2 months (group III) and 15 obese diabetic subjects (group VI). Serum insulin, leptin, adiponectin and resistin were measured by ELISA. Lipid profile was measured by a spectrophotometric method. Anthropometric measurements were also performed. The study showed that the obese and obese diabetic subjects have got higher serum leptin and resistin levels when compared with controls. In contrast, serum adiponectin concentration was significantly lower in obese before diet and obese diabetic subjects when compared to the control group. After weight loss, there is an improvement in all these parameters. The findings from bivariate correlation analysis were further explored using multiple linear regression analysis which confirms that resistin rather than adiponectin and leptin was an important determinant of insulin resistance. From all the above work, we conclude that even modest weight loss can improve metabolic risk factors through changes in some cytokines.

**Abstract Text**

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**Keywords:** Analysis, Biological Samples, Biomedical, Data Analysis

**Application Code:** Biomedical

**Methodology Code:** Chemical Methods
Aflatoxins are extremely carcinogenic mycotoxins produced by notorious Aspergillus flavus and Aspergillus parasiticus. AFB1, AFB2, AFG1 and AFG2 are the primary secondary metabolites, which are generally produced by aflatoxigenic fungi. Among these, AFB1 is the most potent organic compound that affects human liver. Prolong exposure to this harmful natural compounds can cause serious mutation in the DNA. In our analytical study, we summarized the role of some biological enzymes which oxidize the AFB1 to AFB1-8,9-exo-epoxide under the effect of cytochrome P450 enzymes, which is highly toxigenic reactive agent. The AFB1-8,9-epoxide is primarily responsible for severe carcinogenicity that cause great mutational alteration in DNA. The preventive majors should be taken to reduce the effect of aflatoxins. Chlorophyllin is a water soluble derivative of chlorophyll found in green plants and vegetables. This molecule potentially blocks cytochrome P450 and reduces the risk of liver cancer. The molecule binds to the aflatoxins, which are present in contaminated food and it can restrict liver infection. This molecule is liable to be used as liver anti-cancer compound.
In recent years, nanodiamonds have been studied for their applications in biology and medicine due to their low cost, broad range of surface chemistry, and relatively small size. Their uses have been explored in bone tissue engineering and drug delivery, and their applications appear promising. However, a full and complete understanding of their toxicity in the human body is required before their applications become practical. Although the various in vivo and in vitro studies seem mostly promising about the toxicity of nanodiamonds, few have been studied on the various functionalized types of nanodiamonds, and almost no study has been conducted to analyze the cytotoxicity of nanodiamonds over various time points in order to get an in-depth view of their toxicity. In this project, we have investigated the cytotoxic effect of different types of nanodiamonds at different doses, and different exposure times by using adenocarcinomic human alveolar basal epithelial cells (A549 Cells). In our experiments, four differently functionalized nanodiamonds at 50 to 250 µg/ml have been dosed to A549 human lung cancer cells with exposure times ranging from 6 to 72 hours. Very interesting results have been obtained through our study. The detailed experimental procedures and results will be presented at the conference. Our study results will be very helpful to the researchers who use nanodiamonds for biomedical applications.

Keywords: Biological Samples, Biomedical, Spectroscopy, Wet Chemical Methods
Application Code: Biomedical
Methodology Code: UV/VIS
The ultimate goal of our research is to develop the so-called bioscopic anticancer chemotherapy with superior tumor response and minimum side-effects even at a greater drug loading concentration. Lipobeads (lipogels) is a good candidate for the conceptually new drug delivery system with release and activation of anticancer agents regulated by bio-signals. Currently, our faculty-student research team focuses on the following directions of study: (1) effect of lipid bilayer fluidity on formation of lipobeads and their application properties; (2) technological aspects of lipobead preparation by polymerization within giant lipid vesicles and nanogel/liposome mixing; (3) lipobeads with “thermophilic” hydrogel core to provide new mechanisms of controlled drug release. Recently, the fluorescent giant vesicles (~10–100 um) were synthesized by gentle hydration of hybrid agarose/lipid films. The giant lipobeads were prepared by polymerization within the giant vesicles. Their morphology and structure were studied by confocal microscopy to find that intactness and stability of the lipid membrane were governed by temperature and bilayer composition, namely: the most ordered (rigid) phospholipid with cholesterol formed a smooth and strong spherical bilayer around the microgel. The poly(acrylamide-co-acrylnitrile) hydrogel was synthesized by photo- and thermal polymerization using different initiators. The amount of cross-linker was varied to enhance the swelling ratio of hydrogel upon heating (positive volume phase transition) in the range of physiological temperatures. Further increase in swelling ability of the hydrogel core loaded with drug modelling molecules and mechanisms of the cargo release at different temperatures are the next steps towards the bioscopic lipobeads.

Keywords: Biopharmaceutical, Drugs, Imaging, Nanotechnology
Application Code: Biomedical
Methodology Code: Microscopy
Cancers are known to produce unique biomolecules or biomarkers that can be detected using diagnostic tests. CA-125 and HE4 are two biomarkers used in clinical tests to detect ovarian cancer, which responds favorably to treatment when diagnosed at its earliest stage. These tests however, often produce both false positives—identifying cancer when it is not present—and false negatives—failing to detect biomarkers that are present. A diagnostic test based on recognition by nucleic acid aptamers may help improve detection of these biomarkers. SELEX is one method used to select aptamers, short oligonucleotides, (usually RNA or single stranded DNA) that bind to a target with high affinity and specificity. We use Ni-NTA coated magnetic beads that bind 6His- HE4, allowing for an easy, bench top separation. This magnetic SELEX approach was combined with a novel fluidics based washing step, allowing for continuous washing of the magnetic beads. Extensive washing removes loosely bound DNA, allowing aptamers with the highest affinity to remain bound to HE4. Using asymmetric PCR we preferentially amplify one strand of DNA, allowing aptamers with the highest affinity to remain bound to HE4. After purification, ssDNA is available for use in subsequent SELEX processes. We successfully completed 5 rounds of SELEX using this approach and will report in this presentation on the affinity properties of the selected aptamers, which are currently being characterized by next-generation sequencing.

Keywords: Bioinformatics, Capillary Electrophoresis, Electrophoresis, Nucleic Acids
Application Code: Bioanalytical
Methodology Code: Separation Sciences
Amperometric Measurements of the Effects of Polyunsaturated Fatty Acids on Exocytosis and Total Vesicle Content in PC12 Cells

We chose to investigate the fatty acids, which have been implicated in learning deficient hyperactive children previously, as they can alter membrane lipid composition and might subsequently effect on neurotransmission. The effects include that lipids which fit in high curvature of membranes facilitate exocytosis and lipids which fit in flat sections of membranes slow exocytosis. We think this might ultimately change synaptic strength and plasticity. Alpha-linolenic acid and linoleic acid are the two essential fatty acids that cannot be synthesized in the body. Therefore, we chose to study the role of these fatty acids on exocytosis and total vesicle content.

Pheochromocytoma (PC12) cells were used to investigate the effects and mechanism of fatty acids on exocytosis and vesicular content. The results obtained from single cell amperometry showed that both essential fatty acids significantly decreased the monoamine amount being released. Moreover, from IVIEC, the total neurotransmitter amount in the vesicles was observed to decrease significantly upon incubation with fatty acids. Only part of the vesicle load of transmitter is typically released and this fraction did not change after treatment with the fatty acids. Therefore, these analytical measurements indicate that addition of these fatty acids influence exocytosis by altering the transmitter content.

Keywords: Electrochemistry, Lipids, Neurochemistry
Application Code: Neurochemistry
Methodology Code: Electrochemistry
Neurochemistry Capillary Electrophoresis

How Electrode Geometry and Material Affect Electrochemical Measurements at Single Cells: Not all Sensors are Created Equal

Amperometry coupled to carbon-fiber disk microelectrodes has proven to be a powerful tool for measuring real-time chemical dynamics at single cells over the last two decades. Many electroanalytical techniques and sensors for bulk analysis have become more sophisticated; however, the methods used for monitoring exocytosis at individual cells in culture have remained largely unchanged. In recent years, our group has made advances in fabricating several new electrodes with novel geometries and materials, showing beneficial electrochemical properties while maintaining the spatial resolution needed to study exocytosis at single cells. These include plasma-etched cavity microelectrodes and carbon nanotube-yarn disk electrodes, among others. The fabrication and potential advantages of each sensor will be described. We have found that even under similar physiological conditions, our advanced sensors can detect more molecules per release event when compared to disk electrodes. This suggests that some of the neurotransmitter released from chromaffin cells goes undetected when using traditional disk electrodes. These findings will provide valuable information to yield a more complete picture of the mechanisms involved in the detection of exocytotic events, offering new information that could potentially improve models of quantal release going forward.

Keywords: Electrochemistry, Microelectrode, Neurochemistry, Voltammetry
Application Code: Neurochemistry
Methodology Code: Electrochemistry
Zwitterionic polymers have recently been demonstrated in biotechnological applications as protein stabilizers and antifouling surfaces. The mechanism of the properties of charged zwitterionic polymers that lead to these applications is hypothesized to be different from the “gold standard” polymer of nonionic, water-soluble polyethylene glycol (PEG). Previous studies have shown PEG weakly binds to proteins in solution and can function like a chaperone that stabilizes the protein, preventing protein aggregation. Similar studies of zwitterionic polymer-protein interactions in solution are lacking. Here, we study the effect of poly(sulfobetaine) on the thermodynamic stability and kinetics of protein folding using temperature-dependent tryptophan fluorescence and circular dichroism measurements. We observe that poly(sulfobetaine) interacts with and destabilizes proteins at high ionic strength. The degree of interaction of poly(sulfobetaine) with proteins is both ionic strength and protein dependent. We compare our results with zwitterionic polymers to other common bulk polymers: PEG and Ficoll. Further, studies of commonly used fluorescent proteins, mCherry and AcGFP, show that poly(sulfobetaine) effects the temperature-dependent quantum yield response of the fluorescence of the proteins by locally effecting the chromophore.

Keywords: Biopharmaceutical, Biospectroscopy, Laser, Protein
Application Code: Polymers and Plastics
Methodology Code: Fluorescence/Luminescence
Polymers and Plastics

Interfacing Liquid Chromatography Methods with Silicon Photonic Microring Resonator Array Detection for Industrial Polymer Analysis

Synthetic polymers are structurally heterogeneous comprising overlapping distributions in molecular weight, chemical composition, topology, and functionality. For copolymers, separation according to chemical composition is typically performed to determine the chemical composition distribution of a sample. Gradient elution liquid chromatography is the technique of choice for such separations. However, challenges exist for quantitative determination of mass concentration with conventional gradient elution detectors. Evaporative light scattering and charged aerosol detectors, while being applicable to gradient elution, both suffer from non-linear concentration responses making quantitative measurements difficult. Although UV detectors can be used for concentration detection in gradient liquid chromatography, many copolymers lack UV or visible chromophores. Silicon photonic microring resonator arrays can be used as detectors in gradient elution chromatography and can serve as quantitative mass concentration detectors in such applications. Microring resonators offer refractive index (RI) based detection with an extended dynamic range, broad solvent compatibility, and linear mass concentration detection. The linear mass concentration detection of the silicon photonic microring resonator was demonstrated with isocratic separations using polystyrene standards with gel permeation chromatography. Gradient separations by adsorption chromatography were applied to poly (methyl methacrylate) and poly (styrene-co-methyl methacrylate) copolymer blends. With these experiments demonstrate the wide dynamic range and gradient compatibility of the microring resonator systems, which could have broad applicability for polymer analysis.

Keywords: Array Detectors, HPLC, HPLC Detection, Polymers & Plastics
Application Code: Polymers and Plastics
Methodology Code: Liquid Chromatography
Session Title: Polymers and Plastics
Abstract Title: A Novel Screening Workflow using Ion-Mobility-Mass Spectrometry for the Analysis of Extractable and Leachable Components from Common Packaging Material

Primary Author: Jane A. Cooper
Waters Corporation

Co-Author(s): Baiba Cabovska

Abstract Text:
Characterization of packaging, food contact materials, medical devices and many other consumables used in various industries is becoming more and more important due to ever increasing global regulations. The initial step in characterizing extractables from packaging includes targeted screening. Which is a well-established process, performed using analytical techniques ranging from GC-FID-MS to LC-UV-MS. The structural elucidation of impurities in the final packaging is typically a very complex and time consuming process. Typically, in identification of compounds, retention time, accurate mass, and fragmentation ion information is used. However, if different chromatographic methods are used, the retention times might vary. If the compound is present at trace levels, the fragments might be absent. If isomers are present, accurate mass as an identification point will not be valid. In this work we demonstrate how using collisional cross section (CCS) values, as an additional data point, acquired using ion mobility-mass spectrometry, can provide increased confidence in compound identification.

In addition this work will also demonstrate a simple workflow that includes scientific library creation, multivariate statistical analysis, elucidation and reporting. Using a single platform informatics solution which can facilitating the decision making process, by aiding analysts in the evaluation of complex data in a more efficient way through simplifying data review.

Keywords: Liquid Chromatography/Mass Spectroscopy
Application Code: Polymers and Plastics
Methodology Code: Liquid Chromatography/Mass Spectrometry
DART®-MS is a powerful method for rapidly analysis. To expanding the capability on it, we had been developed two devices, a thermal desorption and pyrolysis device; ionRocket, and an enhancing device for volatile compounds device; Volatimeship. We’ll describe some analysis applications.
Optical Methods for Evaluation and Characterization of Gelatin Biodegradable Films with Red Propolis and Essential Oil as Additive

Gelatin is a protein with the ability to form a tridimensional network, with crystalline intermolecular linking zones. These compounds are used to produce biodegradable films due to ability to carry active compounds. The aim of the research was evaluation of based on gelatin biodegradable films respect to optical properties. Gelatin films using red propolis (RP), essential oil of basil (BEO) and clove (CEO) as additives using spreading methodology. Nine formulations in different proportions were prepared. Light barrier properties were registered in transmittance. CIELab parameters for Luminance (L), red/green, blue/yellow chromaticity (a*, b*), opacity (Opa) and brightness (Bns 60°) were evaluated. Surface and structural properties were studied under scanning electron microscopy (SEM), atomic force microscopy (AFM) and X-ray. Statistical evaluation performed by ANOVA analysis. (RP), get incidence in transmittance and properties of light barrier, red color is characteristic. Incorporation of (BEO) and (CEO) show difference respect to the control film near to 300nm, with significance different between treatments, typical values in the control film for (L) was 89.9±0.4, (a*) -1.6±0.1 and (b*) 5.9±0.3, that are highest between the values for (L) in formulations (72.7±0.7 to 75.6±0.6), but different for (a* and b*) 12.9±0.5 to 16.7±0.3 and 29.0±0.5 to 36.1±0.9 respectively; characteristic color changes from 5.8±0.4 to 53.7±0.6. (Opa) property (3.9±0.1) were registered in control, highest values depend on (BEO) addition; (Bns 60°) measurements is in relations to gelatin (125.8±0.6 in control film) and 96.3±0.4 to 115.5±0.5. Surface and roughness properties were heterogeneous, depended of additive compounds mainly (BEO). No structural modification in crystalline gelatin and elaborated films were detected in X-ray. Instrumental analysis provided important tools in characterization and study of biodegradable films.

Keywords: Atomic Force Microscopy (AFM), Spectroscopy, Surface Analysis, UV-VIS Absorbance/Luminescence
Application Code: Polymers and Plastics
Methodology Code: Microscopy
Gelatin is a biopolymer with excellent film-forming properties; physical and functional characteristics depending on the gelatin origin, plasticizer, additives and drying conditions. The aim of the research was the characterization mechanical, structural and physico-chemical of gelatin-based biodegradable films. Nine gelatin-based films formulations were produced using red propolis (RP), essential oil of basil (BEO) and clove (CEO) as additives, using spreading methodology. Mechanical properties were evaluated for tensile strength (TS), elongation at break (EB) and elastic modulus (EM). Solubility in water (S), moisture content (M) and water vapor permeability (WVP) were estimated as physico-chemical parameters. Hydrophobicity of films was determined through the contact angle (CA). Identification of functional groups by Fourier transform infrared spectroscopy (FTIR). Statistical evaluation performed by ANOVA analysis. Addition of (RP) and (EO’s) had direct influence of properties biodegradable films. The thickness showed variation between 0.072±0.001 to 0.088±0.002mm. Values of (TS) and (EM) decreased in relation of control film (41.0±1.1 to 19.3±0.9MPa) and (7.6±0.2 to 3.4±0.1MPa) respectively. Variations in (EB) were attributed mainly to (CEO) and (BEO) with values between 75.4±2.5 to 37.5±1.5%. Although the addition of (RP) and (EO’s) presented significant variations between the values of (S), (M) and (WVP), technologically these properties remained almost the same, but, these compounds produced a decrease in (CA) values the side in contact with the air in relation to the control film (87±1.5 to 66±1.0), which suggests an increase in the hydrophobicity of the films. No structural modification in functional groups of organics and inorganics compounds in biodegradables films were detected in FTIR analysis. The addition of (RP) and (CEO-BEO) modified some of the mechanical, physical and chemical properties and structure of biodegradable films.
Electrochromism is a unique property of some conjugated polymers. Analytical applications of electrochromic polymers was reported in our previous work [1]. However well pretreatment of electrode surface is important for formation of stable conjugated polymer layer [2], because not all conjugated polymer films are stable if deposited on not well pretreated surface [3]. The aim of this research was to evaluate electrochromic properties of several different electrochemically deposited conjugated polymer and copolymer layers, which were based on polyaniline (PANI), polypyrrole (Ppy) and some other conjugated polymers. The influence of conducting polymer synthesis conditions, concentrations of monomers, applied potential profile on properties of formed electrochromic layers were investigated. Most efficient electrochromic systems based on PANI, Ppy and other conjugated polymer layers was determined. Spectroscopic properties of formed polymer layers were investigated. The influence of nature and composition of substrate on which electrochromic layer were deposited was evaluated. Stability of formed electrochromic layers was determined. Mechanism of electrochromism will be discussed. Applicability of formed electrochromic layers in analytical devices will be outlined.

Acknowledgement: Research was funded by a grant (No. S-LAT-17-1) from the Research Council of Lithuania.

References

Keywords: Electrochemistry, Electrodes, Semiconductor, Sensors
Application Code: General Interest
Methodology Code: Electrochemistry
Protein kinases regulate cellular processes such as cell growth, metabolism, proliferation, and differentiation. Kinases are enzymes that catalyze phosphate transfer from adenosine triphosphate (ATP) to their target substrates. In this process ADP and phosphorylated substrates are produced. We used SRC kinase to phosphorylate its peptide substrate (KVEKIGETYVYVYK-amide) with ATP$^{33}$P, to evaluate SRC kinase activity and make a sensor for ATP and SRC substrate.

Radioisotopes are used in bioanalytical measurements as they provide the lowest perturbation on analyte properties, such as binding kinetics. $^{33}$P isotope has higher decay energy than $^3$H and the resulting beta particles travel a longer distance (ca. 600 $\mu$m in water), making $^{33}$P a better tracer for sensitive detection of phosphorous-containing analytes, such as $^{33}$P-ATP, or $^{33}$P-labeled analytes, such as phosphorylated substrates.

In this research, polystyrene-silica core-shell scintillating nanoparticles doped with reporter fluorophores (pTP and DMPOPOP) were fabricated for scintillation proximity assay (SPA). SPA works based on the conversion of energy released from bound radiolabeled analyte to detectable visible light. Thiol-functionalized nanoparticles were used to immobilize $^{33}$P-phosphorylated SRC substrate on the surface of nanoparticles, with the aid of a sulfhydryl to amine crosslinker, i.e. Mal-PEG2-NHS ester. This study shows that scintillating nanoparticles may be used for evaluation of kinase activity and as sensors of ATP in picomolar concentration.

**Keywords:** Bioanalytical, Biosensors, Nanotechnology, Radiochemical Methods

**Application Code:** Bioanalytical

**Methodology Code:** Sensors
There is a great interest in better understanding the phenomena that occurs at the nanoscale between metallic/catalytic nanoparticles and hydrogen gas. This has implications not only in fundamental aspects but also in some applications such as in hydrogen sensing and catalysis. We chemically synthesize Pd, Pt, and Pd/Pt alloy nanoparticles (NPs) protected with organic ligands which are later assembled on graphene (obtained by CVD). The film of NPs is simply formed by incubating graphene in the NPs solution for some time. Once the heterojunction is formed, it is placed in an ad-hoc chamber and exposed to alternating flows of pure He and H2 (at 5%) during in-situ GISAXS experiments. First, I will show the differences in NPs assembly when using Si vs. graphene as substrates. I will also show how the film restructures upon the presence of H2 gas and the importance of the metal composition. Finally, I will demonstrate that this heterostructures can be used for detecting H2 at low concentrations and for reducing molecules such as methylene blue.

Abstract Text

Primary Author
Francisco Ibanez
CONICET-University of La Plata

Co-Author(s)
Maria C. Dalfovo

Keywords: Sensors
Application Code: Nanotechnology
Methodology Code: X-ray Techniques
Can Electrospray Ionization-Mass Spectrometry be Used to Predict the Potentiometric Selectivity of Neutral Carrier Ionophores?

Electrospray ionization mass spectrometry (ESI-MS) is a powerful technique for the characterization of macromolecules and their noncovalent binding with guest ions. In this presentation, we demonstrate the feasibility of using ESI-MS as a screening tool for predicting potentiometric selectivities of neutral carrier ionophores. The cyclic peptide, cyclosporin A, was used as a model new ionophore in plasticized poly(vinyl chloride) membranes. Optimized membranes demonstrated near-Nernstian slopes with micromolar detection limits toward calcium. ESI-MS and ESI-MS/MS were employed to determine the relative association strengths of cyclosporin A with various cations. The observed MS intensities of ion-ionophore complexes correlate favorably with the potentiometric selectivity pattern that was demonstrated by cyclosporin-based electrodes. This correlation was found to hold true for other established ionophores, such as valinomycin and benzo-18-crown-6. Furthermore, competitive-binding selectivity in a mixture of ionophores with various cations was demonstrated as a model for screening combinatorial library of receptors. Taken together, these experiments demonstrate that mass spectrometry could be used to predict the selectivity patterns of new ionophores for potentiometric and optical ion sensors. Further, this approach could be useful in screening mixtures or libraries of newly synthesized compounds to identify selective ionophores.

Keywords: Ion Selective Electrodes, Mass Spectrometry
Application Code: General Interest
Methodology Code: Sensors
Solid contact ion-selective electrodes (SC-IS) are multilayer structures, which have an intermediate layer, e.g. conductive polymer or carbon layer, between the ion-selective membrane and the electron conductor substrate electrode. One of the main concerns with SC-IS is a formation of an aqueous film ("water layer"1) between this intermediate layer and the ion-selective membrane which is considered the source of drifting potentials, poor potential repeatability and reproducibility. Fibbioli et al. developed simple test to check existence of water layer1. However, this test is very time consuming and the interpretation of the results is often not straightforward. For example if the experimental conditions were not selected appropriately it might give false negative result2, i.e., lead to improper conclusion. Recently, it has been shown that the “water layer test” can be performed in minutes for SC-IS with pH-selective membrane2 through the assessment of the CO2 sensitivity of the electrode. However, if the aim of the “water layer test” is to gain information on its detrimental effect in specific experimental condition one should consider that: (i) The length of the experiment depends on the membrane thickness and the diffusion coefficients; (ii) The test should be performed with membranes with limited selectivity for the tested ion.

In this work we show examples how the different factors (like membrane thickness, membrane selectivity, length of experiment, water layer thickness etc.), influence the outcome of the “water layer test” and discuss how to design “water layer test” and recommend protocols for unambiguous results.

1 Fibbioli, M.; Morf, W. E.; Badertscher, M.; de Rooij, N. F.; Pretsch, E. Electroanalysis 2000, 12, 1286-1292.
Non-Covalent Surface Modification of Graphene Using Pyrene and Cyclodextrin Derivatives

Despite the interest in recent years on graphene-based chemical sensors, surprisingly little has been reported so far on the details of interaction-driven self-assembly on graphene. With a view to graphene-based sensing arrays and devices, graphene was modified in this work with monolayers of ten pyrene and cyclodextrin derivatives as receptors. The receptor compounds were pyrene, pyrene derivatives with hydroxyl, carboxyl, ester, ammonium, amino, diethylamino, and boronic acid groups, as well as the perbenzylated α-, β-, and γ-cyclodextrins. Adsorption of these compounds onto graphene was quantified by contact angle measurements and X-ray photoelectron spectroscopy (XPS). Data thus obtained were fitted with the Langmuir adsorption model to determine the equilibrium constants for surface adsorption and the concentrations of self-assembly solutions needed to form dense monolayers on graphene. The equilibrium constants of all pyrene derivatives fell into the range from $10^{3.4}$ to $10^{4.6}$ M$^{-1}$. For the perbenzylated α-, β-, and γ-cyclodextrins, the equilibrium constants are $10^{3.24}$, $10^{2.97}$, and $10^{2.95}$ M$^{-1}$, respectively. Monolayers of 1-pyrenemethylammonium chloride on graphene were confirmed to be stable under heating up to 100 °C in a high vacuum (2×10$^{-5}$ Torr), and monolayers of 1-pyrenemethylamine can be removed from graphene by immersion into toluene.

Keywords: Adsorption, Sensors, Surface Analysis
Application Code: Bioanalytical
Methodology Code: Sensors
Determination of cell growth and viability is crucial for bioprocesses monitoring. Traditionally, the control of the cell culture growth is performed by cell counting, which can be a time-consuming procedure that requires an expert user. Thus, in order to take the cell culture control to the next level, electrochemical enzymatic sensors for the in-situ measurement of substrates and metabolites of interest in cell cultures were developed.

Two of the most commonly monitored parameters are glucose, as the major carbon and energy source, and lactate, as a metabolic product. Therefore, two amperometric enzymatic sensors specific towards this key analytes were developed. Glucose and lactate sensors were based on screen-printed miniaturized technology which allowed collecting samples from the media in order of microliters, without disturbing the cell culture. The proposed glucose and lactate sensors were able to distinguish differences in glucose and lactate levels in cell media 24 h after the cells have been seeded. Due to the quantitative establishment of the uptake of glucose and production of lactate it was possible to effectively control the growth process stages. Aspects such as the limits of detection, reproducibility and repeatability were studied and the figures of merit were obtained.

The developed sensors revealed to be robust, highly stable, user-friendly and time-saving, demonstrating the possibility of being used as a cell culture monitoring trustful tool.

Acknowledgements
This work was supported by the Project “BIOTEAR” (IDE/2016/0000216), co-financed through IDEPA and ERDF fund.

Keywords: Bioanalytical, Biosensors, Chemically Modified Electrodes, Portable Instruments
Application Code: Bioanalytical
Methodology Code: Sensors
Abstract Text

We studied the optical properties of gold nanorods (AuNRs) using a dark-field (DF) microscope and a scanning electron microscope (SEM). The existing LSPR based biosensor was a method of sensing by the shift of the LSPR peak when the target molecules were attached. However, the dielectric constant of the surrounding medium affects this sensing method. We therefore propose a label-free plasmon-based biosensor that is sensitive to the adsorption of biomolecules without being disturbed by the dielectric constant of the surrounding medium. When thiol molecules are attached to AuNRs, LSPR wavelength shifts and a full width at half maximum (FWHM or LSPR linewidth) is broadened due to the chemical interface damping (CID) effect. In the present study, we investigate the effect of size of AuNRs on the broadening of the LSPR linewidth by the CID effect with three different aspect ratios. Furthermore, we demonstrate a LSPR biosensing method based on the change of the LSPR linewidth by the adsorption of thiol molecules.

Keywords: Adsorption, Biosensors, Microscopy, Spectroscopy

Application Code: Bioanalytical

Methodology Code: Sensors
Electrochemical detection is particularly useful in neuroscience because it enables researchers to study neurochemical dynamics by making real-time measurements. However, non-electroactive species, such as glucose, require biosensors which are stable, selective, and have physiologically relevant sensitivities to targeted analytes. We have demonstrated sub-second electrochemical detection of glucose fluctuations by combining glucose oxidase-modified carbon-fiber microelectrodes with fast-scan cyclic voltammetry. The sensing surface of the microelectrode is modified with glucose oxidase to create biosensors sensitive to glucose. Glucose oxidase enables the electrochemical detection of glucose through the production of hydrogen peroxide, which is electroactive and serves as the glucose reporter molecule. Work presented herein quantitatively compares three approaches for enzyme immobilization - physical adsorption, hydrogel entrapment, and electrospinning. The enzyme is immobilized on a carbon-fiber microelectrode that can be coupled with voltammetry and implanted in spatially discrete locations of the brain. The data suggest that each of these methods can be used to create functional microbiosensors. However, hydrogel entrapment is the most effective approach to glucose oxidase immobilization on the carbon electrode surface when detecting physiological concentration of H[2]O[2]. This finding should be broadly applicable to other enzymes, and thus promises to provide an effective means for researchers to study topics in neuroscience involving the detection of a variety of non-electroactive molecules.
Fatigue and drowsiness are known to affect safety and performance in the workplace. It is well established that workplace injuries correlate with worker alertness and fatigue. Moreover, even modest levels of fatigue can mirror the impairment caused by alcohol intoxication as it relates to work safety. This highlights the importance of identifying biomarkers in breath to ensure proper identification of drowsiness. To identify the potential breath biomarkers, we performed a study where we investigated the effect of fatigue and drowsiness on human metabolism by collecting the exhaled breath of resident physicians before and after work. We aimed at determining a correlation between the contents of their breath profiles and the ratings of their levels of drowsiness, stress, and fatigue. Using Field Asymmetric Ion Mobility Spectrometry (FAIMS) followed by GC-MS, we identified 13 volatile organic biomarkers for drowsiness. We then developed miniaturized solid state sensors for these biomarkers and employed them to detect them in the breath of our human subjects. After verification of the validity of our sensors for detection of the biomarkers in breath, we simulated a real situation of breath biomarker detection for drowsiness as a safety function within an automobile. For that, we employed an array of solid state sensors capable of competently detecting the volatile organic breath biomarkers identified as predictors of stress, fatigue, and drowsiness. Specifically, sensor efficacy was tested by releasing the volatile target biomarker within a car environment with a capacity of roughly 1500 liters of volume. Our solid state sensor array was able to successfully detect the biomarker at the concentration levels exhaled by humans, thus successfully demonstrating the potential of our technology to determine a driver’s physiological state behind the wheel.

Keywords: Gas Chromatography/Mass Spectrometry, Material Science, Specialty Gas Analysis, Volatile Organic
Application Code: Biomedical
Methodology Code: Sensors
Abstract Text
This talk will cover the full scope of nanobiosensing, which combines the newest research results in the cross-disciplines of chemistry, biology, and materials science with biosensing and bioanalysis to develop novel detection principles, sensing mechanisms, and device engineering methods. It not only covers the important types of nanomaterials for biosensing applications, including carbon nanotubes, carbon nanofiber, quantum dots, fullerenes, fluorescent and biological molecules, etc., but also illustrates a wide range of sensing principles, including electrochemical detection, fluorescence, chemiluminescence, antibody-antigen interactions, and magnetic detection.

The talk details novel developments in the methodology and devices of biosensing and bioanalysis combined with nanoscience and nanotechnology, as well as their applications in biomedicine and environmental monitoring. Furthermore, the reported works on the application and biofunction of nanoparticles have attracted extensive attention and interest, thus they are of particular interest to readers. This talk also will discuss nanobiosensing technology, including the principles and application of biosensing, the design and biofunctionalization of bionanomaterials, as well as the methodology to develop biosensing devices and bioanalytical systems.

Keywords: Biological Samples, Biomedical, Biosensors
Application Code: Biomedical
Methodology Code: Sensors
The development of user-friendly, time-saving and cost-effective analytical strategies for the detection and quality control of carbohydrates in food industry is continuously highly demanded. Therefore, in this work, two strategies for the non-enzymatic detection of reducing sugars (glucose and fructose), employing miniaturized screen-printed gold electrodes, were proposed.

On the one hand, a high-throughput flow-injection analysis (FIA) system that employed gold electrodes integrated in a one channel flow-cell was developed. The injection of sample volume (60 µL) was done through an “in-line luer” injection port, placed closest to the electrochemical cell, and highly controlled by operator through a syringe. This configuration brings important advantages since it simplifies operability and effectiveness of working in FIA systems. The sugars detection was carried out by applying a potential of + 0.5 V in a NaOH 0.1 M flow carrier stream of 1 ml min⁻¹. On the other hand, a batch mode approach using disposable gold electrodes for sugars detection by dropping the sample (60 µL) directly in the electrochemical cell was also investigated. The analytes were measured amperometrically, by applying a constant potential of + 0.5 V during 2 min, and by determining the anodic oxidation peak current by cyclic voltammetry.

Accuracy and precision studies were performed and the limits of detection and linear ranges were established. Finally, the applicability of the developed non-enzymatic sensors has been successfully evaluated by determining reducing sugars in commercial foodstuffs.

Acknowledgements
This work was supported by the MANUNET ERA-NET Project “SENS4WINE” (IDE/2016/0000157), co-financed through IDEPA and ERDF fund.

Keywords: Carbohydrates, Electrode Surfaces, Portable Instruments, Sensors
Application Code: Food Science
Methodology Code: Sensors
Diabetes is a metabolic disease that affects approximately 10% of the population in the United States. Research into diabetes has primarily focused on insulin secretion, but recent studies suggest that hyperglucagonemia, the over-secretion of the signaling peptide glucagon, may play a crucial role in development or progression of the disease. Methods to better characterize glucagon secretion would significantly benefit the field, as this process is poorly understood. Electrochemical methods utilizing a microelectrode will provide us with an analytical technique to directly detect the secretion of glucagon from individual cells with high temporal resolution. Historically, carbon fiber based microelectrodes have been used for monitoring small molecule and hormone secretion from cells. One issue with these electrodes is that they are susceptible to surface fouling, which reduces signal intensity and reproducibility. To overcome this problem, modification of the surface of the electrode have been utilized.

In this research, the electrodes are modified with carbon nanotubes and chitosan to reduce fouling while maintaining adequate temporal resolution to study the exocytotic events. The concentration of chitosan tested ranged from 0.00 to 0.20% w/w while the concentration of carbon nanotubes ranged 0.0 to 1.5 mg/mL in 0.10M HCl. Electrodeposition and dip coating have been explored to determine optimal conditions to produce reliable and stable electrodes with excellent temporal resolution. These modified microelectrodes have been successfully utilized to detect tryptophan and tyrosine, electroactive residues found in glucagon, at concentrations ranging from 1-20 [micro]M. Current efforts are focused on the detection of glucagon in the presence and absence of commonly secreted islet hormones, with the ultimate goal of detecting exocytotic events from single-cells to better characterize glucagon secretion.

Keywords: Amino Acids, Electrochemistry, Electrode Surfaces
Application Code: Bioanalytical
Methodology Code: Electrochemistry
Escherichia coli (E. coli) are a dangerous pathogenic bacteria, and it is necessary to quickly detect E. coli in low concentrations. When E. coli and silver nanoparticles (AgNPs) interact in solution, AgNPs bind to the bacteria. Once the bacteria are coated in AgNPs, they can be electrochemically detected by direct oxidation of the AgNPs at the surface of an indicator electrode. Compton group has detected AgNP-covered E. coli on a microelectrode at room temperature. In this work, a high frequency ac waveform is used to heat a microelectrode. This incites electrothermal fluid flow (ETF), and a dielectrophoretic force (DEP) in solution. ETF and DEP preconcentrate the AgNP-covered E. coli near the surface of the electrode, resulting in an increase in the frequency of collisions. Using these phenomena as a preconcentration step will lower the detection limit for detecting E. coli by silver oxidation. This method not only allows for low detection limits, but is much faster than the current method of plate counting.


Keywords: Detection, Electrochemistry, Microelectrode
Application Code: Bioanalytical
Methodology Code: Electrochemistry
The interactions of single stranded DNA immobilized on gold electrodes with hexammine ruthenium(III) (RuHex) and hexammine cobalt(III) (CoHex) were investigated in order to find the optimal potential jump with electrochemical quartz crystal microbalance (EQCM) experiments [sup][1][/sup]. The modified surfaces were created by immobilizing thiolated ssDNA to form a compact, self-assembled monolayer (SAM) followed by passivation with 6-mercaptop-1-hexanol (MCH). Formation of these modified SAMs using a similar protocol proposed by Steel et.al is shown to produce heterogeneous surface packing densities with unequal distribution and aggregate formation of ssDNA and MCH over the gold surface. Here we have used a modified immobilization protocol that resulted in significantly different SAMs with a much lower surface density and with fewer aggregates. We observed a notable decrease (by $\bullet 10$ Hz) in frequency shift values in the presence of both CoHex and RuHex with the modified protocol. The effect of normal and heavy water on the interaction of the redox species with the two types of SAMs was also studied, and turned out to be quite different with each redox indicator (Fig. 1). Since the viscosity of heavy water is only 20% higher compared with normal water, we believe that these findings indicate an H/D kinetic isotope effect upon the binding of the redox complexes with ssDNA. CoHex is known to influence the secondary and tertiary structure of DNA forming intermolecular bridges between strands. The involved hydrogen bonds can be influenced by kinetic isotope effects leading to the difference in the frequency shift values observed. The new protocol showed a promising way of developing more uniformly distributed SAMs on gold surfaces without aggregation of species which can ultimately lead to better performance on sensor surfaces.


Keywords: Bioanalytical, Electrode Surfaces, Nucleic Acids, Voltammetry
Application Code: Bioanalytical
Methodology Code: Electrochemistry
Acetaminophen is a well-known electro-active compound which shows a complex reaction mechanism depending on the solution’s pH. We studied the influence of temperature on acetaminophen and its oxidation products on gold electrodes using intermediate pH 7.4 and acidic pH solutions. At acidic pH, the oxonium ions catalyzes a consecutive hydrolysis of the quinoidal oxidation product. Around neutral pH, a dimerization happens between the acetaminophen-oxidation product and another acetaminophen molecule which clearly indicates that the Acetaminophen is involved in both consecutive and parallel reactions.

We have compared the electrode kinetics of redox couples at heated electrodes with rotating disk electrode RDE (0-8000 rpm) at 10 mV/s. Koutecky-levich K-L plots were recorded to distinguish the contributions of mass transport & heterogeneous electron transfer. For the heated electrode, it was hypothesized that the two extreme cases of very low & high electrode temperature with slow & quick mass transport would allow insight into the reaction kinetics. For RDE, K-L plots have been recorded to reveal the reaction rate of the electron transfer reaction at infinite mass transport speed Fig.1. At pH 7.4, a complex EC mechanism was studied, and apparently it gives a negative Ea. One possible explanation might be different adsorption tendencies of ions involved. An alternative explanation is that we see here an ln I vs 1/T plot of the Ta equation of the oxidation reaction. Further investigations are underway to clarify this behavior. At pH1, a simple consecutive reaction occurs, does not interfere with the observed electron transfer reaction and gives a positive Ea. At both neutral and acidic conditions the Ea at 500 rpm was at a low level (9-14 kJ/mol) and very similar indicating a diffusion controlled reaction. Experiments with gold RDE at various bulk electrolyte temperatures confirmed the different reaction mechanism of dimerization and hydrolysis.
Hydrogen peroxide (H$_2$O$_2$) has been implicated in the slow destruction of dopaminergic neurons in Parkinson’s disease (PD). This neurodegenerative disease affects more than one million people in America, creating a critical need to identify the mechanisms through which H$_2$O$_2$ interacts with dopaminergic neurons. Real-time detection of this analyte in vivo has recently been described using fast-scan cyclic voltammetry at carbon-fiber electrodes. However, distinguishing H$_2$O$_2$ from interferents such as adenosine and pH shifts remains a challenge. Additionally, chemical agents used to pharmacologically verify the presence of hydrogen peroxide production in the brain, such as mercaptosuccinic acid (MCS), have similar oxidation peaks to that of the target analyte, further convoluting the characterization of robust H$_2$O$_2$ dynamics in the brain. We have addressed these problems by fabricating mechanically robust H$_2$O$_2$ selective electrodes. 1,3-phenylenediamine (mPD) was electrodeposited onto the surface of the carbon-fiber electrode to render it insensitive to larger analytes, including dopamine, MCS, and adenosine. Shifts in pH are detected, but these generate a well-characterized and distinct voltammogram, that can easily be removed from the signal using principal component regression, leaving an electrochemical signal due solely to the oxidation of H$_2$O$_2$. This technology was fully characterized and validated, providing a valuable tool for further elucidation of the neurodegenerative role that H$_2$O$_2$ plays in PD, as well as other neuropathies involving oxidative stress.

Keywords: Chemically Modified Electrodes, Electrochemistry, Neurochemistry, Voltammetry

Application Code: Neurochemistry

Methodology Code: Electrochemistry
In this paper we report the interaction of functionalised carbon nano spheres (FCNSs) with dopamine (DA), Uric acid (UA) and L-ascorbic acid (AA). FCNSs were characterised using SEM, AFM, spectroscopic and electrochemical measurements. Functionalised carbon nano spheres paste electrode (FCNSPE) has shown excellent preconcentration property of DA over its surface. UA also has also shown good electrochemical activity over the modified electrode. Parameters were optimised for the enhanced oxidation signal of DA in connection with simultaneous determination of DA and UA. Corresponding voltammograms are shown in Fig.1. An analytical method was developed for the simultaneous determination of DA and UA using the simple method of fabrication of electrode with detection limits (S/N of 3) of 10 nM and 42 nM for DA and UA respectively. Electrochemical oxidation signals of DA and UA were interference free from the oxidation of AA and other commonly occurring interfering agents. The method was applied for the determination of DA in spiked blood serum sample and for UA in real blood serum samples. Interaction of DA and UA was also carried out using the Re2O3 and FCNSs composite materials modified electrode and as shown in Fig. 1 E and F, this composite material has shown even better electrochemical activity for DA and UA compared to only FCNSs modified electrode.
In this work, a glassy carbon electrode modified with quantum dots of graphene and gold nanoparticles (GCE/GQDs/AuNPs) was developed for the norepinephrine (NE) determination using square wave stripping voltammetry (SWSV). GQDs were synthesized by pyrolysis of citric acid and were characterized by UV-Vis and fluorescence spectroscopy. The AuNPs obtained by chemically synthesis were characterized by UV-Vis spectroscopy (Surface Plasmon Band). The GCE/GQDs surface was characterized by raman spectroscopy. The optimum conditions for the determination of NE with GCE/GQDs/AuNPs were: an accumulation potential of 0.00 V (Eacc), time accumulation of 30 s (tacc), phosphate-buffered saline (PBS) pH 7.0 and frequency of 15 Hz. The linear range was observed between 0.5 to 7.5 \([\text{micro}]\text{mol L}^{-1}\), with a detection limit (LOD) of 0.15 \([\text{micro}]\text{mol L}^{-1}\). The proposed methodology was validated with fortified samples obtaining good precision and accuracy. GCE/GQDs/AuNPs was applied in pharmaceutical preparations (NE ampoules) and in rat brain tissue with satisfactory results.
An IgE Biosensor Based on Polymelamine-modified Screen-printed Carbon Electrodes

We herein report the use of melamine and a low-cost screen-printed carbon electrode (SPCE) as the base matrices for the preparation of an electrochemical biosensor. Following the electrochemical polymerization of melamine, the resulting polymelamine was deposited on the SPCE surface to give layers bearing –NH₂ functional groups, which allowed the attachment of anti-IgE (immunoglobulin E) antibodies. The resulting anti-IgE-labeled SPCEs were then incubated with IgE solutions of various concentrations prior to analysis by chronoamperometry using Ru(NH₃)₆³⁺ as an electrochemical mediator. A logarithmic relationship was observed between the chronoamperometric current and the IgE concentration between 5.3 and 530 fM (i.e. over 2 orders of magnitude). In addition, a detection limit of 0.64 fM was achieved in addition to a recovery of 114 ± 14% for a fetal bovine serum sample spiked with 16 fM IgE. This study represents the first reported use of polymelamine for antibody immobilization on an electrode surface. Furthermore, only a small quantity of sample was required for analysis, and the IgE assay was suitable for use in a complex serum matrix without interference. We therefore expect that this novel system will be useful for monitoring the changes in blood IgE levels during the clinical treatment of allergic asthma and rhinitis.

Keywords: Bioanalytical, Biosensors, Chemically Modified Electrodes, Electrochemistry
Application Code: Bioanalytical
Methodology Code: Electrochemistry
**Abstract Title:** Design, Fabrication and Analytical Application of Multi-Channel Amperometric Flow Cells

**Primary Author:** Sayed A. Marzouk  
United Arab Emirates University

**Co-Author(s):** Aisha R. Al Yammahi, Jody D. Haddow, Muna Bufaroosha

**Abstract Text:**
Design, construction, and application of two 4-Channel amperometric cells equipped with screen printed and conventionally sized electrodes, respectively, are presented. The presented flow cells are designed in such a way to allow (i) consecutive determination of hydrogen peroxide in the same carrier buffer stream, (ii) maintaining minimum dead volume to avoid excessive sample dilution while travelling from one detector to another (D#1-D#4), and (iii) tolerate relatively high upstream pressures since immobilized enzyme reactor will be inserted in line between each two successive detectors. The present system is used in the determination of pyruvate, free sialic acid and bound sialic acid at detectors D#2, D#3 and D#4, respectively, whereas detector number 1 (D#1) is reserved for the evaluation of the interference from oxidizable interfering species. To achieve this task, three immobilized enzyme reactors (IERs) are prepared with (i) pyruvate oxidase (PO), (ii) mixture of PO and sialic acid aldolase (SAA), and (iii) mixture of PO, SAA and sialidase (SD), respectively. These, IERs generate hydrogen peroxide proportional to pyruvate, free sialic acid and bound sialic acid in the injected sample, respectively. The eliminate the carry over effect of the produced hydrogen peroxide after signal registry at detectors number 2 and 3, a small IER containing catalase is inserted immediately after these two detectors and before the following analytical IER. Each detector (whether based on screen printed or conventionally sized platinum disc electrodes) is consisted of 3-electrode cell in which platinum is used as working electrode. The construction details of the novel flow cells and the analytical applicability of the proposed system will be presented.

**Keywords:** Bioanalytical, Biomedical, Electrodes, Flow Injection Analysis

**Application Code:** Bioanalytical

**Methodology Code:** Electrochemistry
Electrodeposition is one of the effective tools for making nanomaterials and fabricating electrode surfaces. In this presentation, we have investigated two different types of electrochemical deposition: one is electrodeposition of tantalum (Ta) in a non-aqueous solution and the other is patterned electrodeposition of metals with scanning electrochemical microscopy (SECM).

Firstly, Ta- was deposited in a non-aqueous solution containing Ta precursor (TaCl₅). Ta has been rarely studied despite its high melting point, toughness, biocompatibility, and corrosion resistance presumably due to the more negative reduction potential of Ta than H₂O and the reactivity of TaCl₅ with moisture. To circumvent H₂O reduction and decomposition of TaCl₅, Ta was electrodeposited in an aprotic solvent, acetonitrile. The Ta deposited electrode was good for oxygen reduction and hydrogen peroxide (H₂O₂) sensing. Especially, we monitored H₂O₂ concentration in real time during catalase or glucose oxidase-involved reactions, either eliminating or producing H₂O₂, respectively. From this kinetic study we evaluated Michaelis constants of each enzyme.

SECM was applied to micropatterning of gold, palladium, and cobalt by electrodeposition. We used microelectrodes made of gold, palladium, and cobalt to generate corresponding metal ions in an aqueous solution by potential control and the generated ions were collected and reduced on substrate electrode, making intended patterns. The resolution of localized deposition depended on the tip size and distance between tip and substrate electrode. Moreover, co-deposition of metals was also possible by optimizing deposition conditions and the variation of local electrocatalytic activities were measured at the interfaces between co-deposited metals.

Keywords: Biosensors, Electrochemistry, Electrode Surfaces, Enzyme Assays
Application Code: Nanotechnology
Methodology Code: Electrochemistry
Glucose sensing has been great interest of the scientific community due to its concentration in biological fluids is correlated with diabetes disease, one of the major global health issues [1]. Due to their importance, several chemical sensors have been developed. Paper-based analytical devices (PADs) represent an alternative to expensive and sophisticated analytical technique because of their attractive features such as low cost, small sample volumes, portable and it can be used easily in remote world places, especially in poorer countries[2]. Electrochemical PADs (e-PADs) can be fabricated combining graphene-based materials and its composites with [i]e.g.[/i] metallic nanoparticles, which provide good electrical conductivity and enhancing electronic transfer [3]. In this work, we present a simple method to fabricate e-PADs with reduced graphene oxide (rGO) and gold nanoparticles (AuNPs) for electrochemical detection of glucose in biological fluids. The first fabrication step of the e-PADs is the wax printing process to build hydrophobic pattern in office paper and consequently to create hydrophilic zone. An aliquot of 4 [micro]L of rGO and AuNPs mixture in 1:1 (v/v) ratio was drop-casted and used as the electronic material to fabricate the working electrode surface and the electrical contact. The reference and auxiliary electrode were Ag/AgCl and graphite mine, respectively. The glucose cyclic voltammogram exhibited oxidation process at low potential (-0.50V) permitting a good selectivity. The analytical curve was plotted using the current signal for the glucose oxidation at -0.20 V and a good linear range is observed between 1 up to 16 mmol L-1. Financial Support: CAPES, CNPq and FAPESP.

REFERENCES

Keywords: Analysis, Bioanalytical, Electrochemistry, Nanotechnology
Application Code: Bioanalytical
Methodology Code: Sensors
Both high sensitivity and high specificity are crucial for detection of miRNAs that have emerged as important clinical biomarkers. Just Another Zinc finger proteins (JAZ, ZNF346) bind preferably (but non-sequence-specifically) to DNA-RNA hybrids over single-stranded RNAs, single-stranded DNAs, and double-stranded DNAs. I will present an ultrasensitive and highly specific electrochemical method for miRNA-21 detection based on the selective binding of JAZ to the DNA-RNA hybrid formed between a DNA capture probe and a target miRNA-21. This enables us to use chemically stable DNA as a capture probe instead of RNA as well as to apply a standard sandwich-type assay format to miRNA detection. High signal amplification is obtained by (i) enzymatic amplification by alkaline phosphatase (ALP) coupled with (ii) electrochemical-chemical-chemical (ECC) redox cycling involving an ALP product (hydroquinone). Low nonspecific adsorption of ALP-conjugated JAZ is obtained using a polymeric self-assembled-monolayer-modified and casein-treated indium tin oxide electrode. The detection method can discriminate between target miRNA-21 and non-target nucleic acids (DNA-DNA hybrid, single-stranded DNA, miRNA-125b, miRNA-155, single-base mismatched miRNA, and three-base mismatched miRNA). The detection limits for miRNA-21 in buffer and 10-fold diluted serum are approximately 2 and 30 fM, respectively, indicating that the detection method is ultrasensitive. This detection method can be readily extended to multiplex detection of miRNAs with only one ALP-conjugated JAZ probe due to its non-sequence-specific binding character. We also believe that the method could offer a promising solution for point-of-care testing of miRNAs in body fluids.
An optical fiber-based surface plasmon resonance (SPR) sensor has been widely applied to immunoassay, DNA hybridization, monitoring of cellular response and so on. Although the optical fiber-based SPR sensor has some attractive advantages, such as small volume of analytes, miniaturization of sensor system and potential capacity for remote sensing, fewer studies have been reported on biosensing applications, compared with conventional prism-based SPR sensor. The reason is probably that it is time-consuming and costly to form ideal thickness of metal thin layer for SPR onto the cylinder-shaped fiber core, precisely.

In this study, a simple and low-cost method for fabricating an optical fiber for SPR sensor was proposed. The method was based on the electroless nickel plating and the subsequent displacement gold plating on the core of the optical fiber. The thickness of the nickel and gold thin films on the core of the optical fiber could be controlled by measuring the reflected light intensity from the tip of the optical fiber during the plating processes. The sensitivity and resolution of the SPR sensor using the fabricated optical fiber, in the refractive index range from 1.333 to 1.348, were found to be 1324.3 nm/RIU and 7.6*10^-4 RIU, respectively. The developed SPR sensor was successfully used in the determination of IgA in human saliva, a marker for human stress. The quantitative values of IgA obtained by the SPR sensor were in excellent agreement with that by conventional enzyme-linked immunoabsorbent assay using a 96-well microtiter plate and microplate reader.
**Abstract Title**: Blue-Emitting Copper Nanoclusters Protected by Thiolated PEG for Sensing pH and Biomolecules

**Primary Author**: Cheng-Ju Yu  
University of Taipei

**Co-Author(s)**: Yung-Cheng Chien

**Abstract Text**
Recently, Cu cluster represent a promising candidate for various application such as bio-imaging and sensor, because the materials costs of the precursors for synthesis of Cu cluster are substantially lower with respect to gold or silver precursors to obtain Au cluster or Ag cluster. However, several synthesized Cu cluster revealed a lack of stability against oxidation at the aqueous surrounding. In this study, we proposed the synthesis of highly fluorescent and strongly stable blue-emitting Cu nanoclusters by chemical reduction of CuSO4 in the presence of thiolated PEG, the polymer of ethylene oxide with thiol chains and the average of molecule weight is 500, and NaBH4 as reducing reagent at 70°C. The as-prepared Cu cluster showed bright blue fluorescence with 30.6% of relative quantum yield in aqueous system and the fluorescence was responsive to pH in that with adjusting the pH from 5 to 7. Furthermore, the thiolated-PEG Cu cluster not only could be stored at room temperature for at least one week, but showing excellent stability in high electrolyte and heavy metals environment, interestingly, and even could resisting against GSH and H2O2. According to above-mentioned properties, we utilized the relationship about the fluorescence of Cu cluster and the pH value of surrounding to design the urea sensor through the reaction of urea and urease to produce the ammonia that causing pH value change. The proposed method could obtain the good linear range from 0.098 to 1.56 mM for sensing urea and LOD is 32 µM.

**Keywords**: Biosensors, Fluorescence, Nanotechnology, Sensors

**Application Code**: Bioanalytical

**Methodology Code**: Sensors
The development of simple, inexpensive and efficient devices for early detection of diseases is needed for significant improvements in the therapeutic outcome. We develop a totally new, low cost, device for detection of cancer biomarker proteins in patient serum for advanced disease diagnostics. The approach employs the capture of biomarker protein from the blood using micron size self-propelled particles (micro-machines). The surface of these micro-machines are functionalized with antibodies that enable them to capture specific protein biomarkers while swimming in the patient sample (Capture on-the-fly). The method may eventually be possible to implement at point-of-care in physicians’ offices. It will also be adaptable to the detection of virtually any diagnostic protein of clinical significance.
Epitope-Resolved Detection of Peanut-Specific IgE Antibodies by SPR Imaging

Peanut allergy can be life-threatening and is mediated by allergen-specific immunoglobulin E (IgE) antibodies. Investigation of IgE antibody binding to distinct allergenic epitopes can more precisely identify the specificity underlying the allergic responses. Here, we describe a 45-min surface plasmon resonance imaging (SPRi) immunoassay for differentiating IgE antibody populations in an epitope-resolved fashion. IgE antibodies are first captured from 10 \( \mu \)L of serum onto magnetic beads bearing IgE \( \beta \)-chain specific antibodies. They are then introduced to an SPRi array equipped with peptide and carbohydrate epitopes from the major peanut allergen glycoprotein \([i]Arachis hypogaea[/i]\) h2 (Ara h2). For the first time, differential epitope responses were achieved by providing a binding environment with conditions that minimized cross-reactivity while maintaining high sensitivity. Excellent correlation was observed between IgE anti-Ara h2 by ImmunoCAP assay results and IgE binding to Ara h2-3 peptide epitope by SPRi results. A weaker correlation was found between IgE anti-Ara h2 by ImmunoCAP assay results and IgE binding to Ara h2-5 peptide and carbohydrate epitopes by SPRi results. These results provide insights into the epitope specificity of peanut-specific human IgE antibodies and the role of immunodominant epitopes and carbohydrates in allergy diagnosis. The analytical approach described here is applicable to future microarrays featuring an expanded cohort of allergen epitopes to achieve a more accurate fingerprint of a patient’s susceptibility and/or severity to peanuts, or, in principle, any other allergens.[i]
Determination of Furazolidone Using a Modified Electrode with a Self-assembly of Cationic Polyelectrolyte-functionalized Graphene Quantum Dots and Gold Nanoparticles

Furazolidone (FZ) is a nitrofuran derivative with chemotherapeutic and antibacterial activity in gastrointestinal infections (Helicobacter pylori) common in animals and humans. In 2014, the FDA banned the use of nitrofurans and other drugs because they proved to be genotoxic and potentially carcinogenic in several cell types. Due to this, it is of great importance to have a fast method for the detection and quantification of this drug.

This work consists in the development of an electrochemical sensor based on a carbon glassy electrode (GC) modified with a cationic polyelectrolyte such as Poly (diallyldimethylammonium chloride) (PDDA) functionalized with graphene quantum dots (GQDs) and gold nanoparticles (AuNPs), for the determination of FZ using square wave voltammetry (SWV).

The GQDs were synthesized from citric acid pyrolysis and were characterized by UV-Vis, infrared (IR) and Fluorescence spectroscopy. The AuNPs were synthesized by chemical reduction and characterized by UV-Vis spectroscopy, obtaining a plasmon band at 520 nm. The electrode surface was modified by the drop-coating technique adding 15 µL of the composite solution (PDDA/GQDs/AuNPs). To avoid the detachment of the composite 5 µL of Nafion were added to the electrode surface.

The optimum chemical and electrochemical parameters for the determination of FZ were: phosphate buffer pH 7.0, accumulation potential (Eacc) = -0.3 V and accumulation time (tacc) = 15 s. Finally, the analytical validation was performed. A linear range of 0.5 - 3.5 M and a detection limit (LD) of 0.06 M was obtained, showing a high sensitivity.

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Nanozyme would be found the different type of enzymes-like activity in the recent year. In this study, we propose a one pot, low cost and earth-friendly synthesis to prepare MnOx/MnFe2O4 nanopopcorns in neutral media which has fructose oxidase- and oxidase-like activity. MnOx/MnFe2O4 was prepared by the reaction between ammonium iron citrate and KMnO4, which was consists of MnOx capped on MnFe2O4 nanoparticle. MnOx/MnFe2O4 nanopopcorns showed high oxidase-like activity, which was confirmed by the oxidation of amplex red to resorufin in the absence of H2O2. However, the activity was highly suppressed in presence of H2O2. Interestingly, the fructose was oxidized by MnOx/Fe2O4 nanopopcorn to produce hydrogen peroxide (H2O2), which can reduce the surface of MnOx to Mn2+, and there is no effect by other sugars, such as glucose, sucrose, mannose etc. leading to the development of a fructose sensor. We studied the kinetics of the oxidase-like activity of MnOx/Fe2O4 in detail and found its high affinity towards the substrates. MnOx/MnFe2O4 nanopopcorn has high selectivity and sensitivity for detection of fructose with a limit of detection of 50 μM.

Keywords: Biosensors, Enzyme Assays, Sensors

Application Code: Bioanalytical

Methodology Code: Sensors
We present a simple lateral flow biosensor strip (LFBS) for the sensitive detection of Human CA 19-9 antigen using gold nanoparticles (GNPs) as colorimetric labels. CA 19-9 is a biomarker that has been associated with pancreatic, colorectal and other gastrointestinal cancers as well as some non-cancerous diseases. The developed LFSB is based on the capture of target CA 19-9 antigen in a sandwich-type assay between an immobilized anti CA 19-9 antibody and GNP- labelled detection antibody. The accumulation of GNP on the LFBS membrane gave a red colored line whose intensity was read with a portable strip reader to quantify CA 19-9. All assay parameters were optimized to give the best sensitivity and reproducibility. The detection limit of the assay was determined to be 5U mL⁻¹ (S/N=3) with a linear dynamic range of 5 U mL⁻¹ to 100 U mL⁻¹. CA 19-9 concentrations in human serum samples were successfully evaluated using the developed assay and the outcome was in accordance with enzyme linked immunosorbent assay (ELISA) results. This shows the potential of the developed assay for the rapid, low cost and sensitive detection of CA 19-9 in clinical samples.
Inflammatory Bowel Disease (IBD) is a group of chronic inflammatory conditions of the gastrointestinal tract (GI) that can cause severe abdominal pain, vomiting, diarrhea, and rectal bleeding. The primary method of diagnosis is colonoscopy with biopsy of pathological lesions, which is invasive and uncomfortable for patients. IBD etiology is not fully understood; among several factors potentially involved it is believed to develop as a reaction to imbalances in the microbial intestinal flora. Bacteria are known to communicate to one another by quorum sensing (QS): quorum sensing is a process of communication based upon small molecules, quorum sensing molecules (QSMs), which regulate of gene expression, including the genes for virulence factors and biofilm formation. As part of ongoing research to identify less invasive means of disease management for IBD, we investigated the levels of QSMs, specifically short chain N-acyl homoserine lactones (AHLs), long chain AHLs, and autoinducer-2 (AI-2), in the serum of IBD patients. During active intestinal inflammation, patients had significantly higher levels of both short and long chain AHLs compared to controls. In contrast, without intestinal inflammation IBD patients had higher levels of only short chain AHLs. Further, levels of AI-2 were comparable between patients and controls regardless of the current state of inflammation. Taken together, these results indicate that AHL levels in serum can serve as a marker for intestinal inflammation in patients with IBD. We believe that this knowledge will be critical in the design and development of non-invasive devices for disease management in IBD and potentially other bacterial related disorders.
The prostate cancer is a worldwide disease with high mortality rate and traditional methods for diagnosis and monitoring are performed through invasive techniques. Currently, the advance of research in medical and biomedical engineering allowed the use of molecular tools combined with nanotechnology to develop portable biosensors specific for major biomarkers to diagnostic, monitoring and treatment of several diseases. More specifically, the cellular responses to biological, chemical or physical stimuli can be evaluated by electrochemical techniques. The objective of this work was to develop integrated biosensors based on aptamers to study different cell biomarkers released by prostate cancer cells, as: free prostatic specific antigen (fPSA), hexokinase type 2 (hK2) and prostatic acid phosphatase (PAP) in a device where the cellular microenvironment can be precisely defined. Briefly, the thiolated DNA aptamer hairpin-shape was conjugated with methylene blue (redox tag), and immobilized on a gold electrode by self-assembly. Binding of specific protein unfold the aptamer pushing MB away from electrode and decreasing electron-transfer efficiency. The change in redox current was quantified using square wave voltammetry (SWV) and three types of prostate cells were evaluated, normal human prostate cells (RWPE-1, ATCC ® CRL-11609™), androgen-sensitive human prostate carcinoma (LNCaP, ATCC ® CRL-1740™) and human prostate adenocarcinoma (PC-3 ATCC ® CRL-1435™) and the aptasensor was able to identify differences on protein released patterns by normal cells and cancer cells with high stability, reproducibility and selectivity offering new insights into the molecular metabolism events.

Keywords: Bioanalytical, Biosensors, Nanotechnology
Application Code: Biomedical
Methodology Code: Sensors
**Abstract Text**

The efficiency of the diagnostic platforms utilizing ELISA technique or immunoassays depends highly on incubation times and volumes of the recognition elements, signaling molecules and the patient samples. In conventional immunoassays, long incubation times and excess amounts of the recognition and signaling molecules are used. The technology proposed here uses electro-mixing of the reagents involved in a sandwich immunoassay based diagnostic kit in such a way that the incubation times and the reagent amounts can be reduced substantially. A diabetes kit for detection of glycated hemoglobin (HbA1c) was chosen as a pilot. The incubation times of the kit was reduced by approximately a factor of 5 when electro-mixing was employed in electrode-enabled microwell plates. Furthermore, if the quantity of the reagents was reduced by half, where almost no distinguishable signals could be obtained with conventional immunoassay, electro-mixing still facilitated acquisition of signals while varying the concentration of the glycated hemoglobin. We found a substantial difference in the signal intensities (up to 166 folds difference) obtained from electro-mixing assisted and conventional immunoassay when the quantity of the reagents and incubation times were kept constant. This technique has a potential to vastly improve the efficiency of immunoassay based diagnostic platforms so that within a very short time and with just sufficient reagents, high throughput analysis of clinical samples may be achieved. It may also open new avenues in point of care diagnostic devices, where kinetics and sampling size/volume play a critical role.

**Keywords:** Bioanalytical, Biomedical, Biosensors, Immunoassay

**Application Code:** Biomedical

**Methodology Code:** Integrated Sensor Systems
As a 2D material with numerous unique properties, graphene has shown great advantages in the application of biological and chemical sensing platforms, featuring small sensor size, label-free detection, and the capability for electronic readout. Herein we describe the development of a graphene-based sensor relying upon the graphene quantum capacitance effect for glucose detection. By creating a graphene variable capacitor (varactor) using microfabrication techniques with pyrene-1-boronic acid (PBA) as the surface functionalization, the selective bonding between PBA and glucose molecules changes the doping sensitive capacitance of graphene, with the potential of in vivo wireless sensing if integrated with an inductor. The PBA surface functionalization on graphene was also characterized using Raman spectroscopy, XPS and capacitance-voltage measurements, evaluating the influence to the device performance. The sensitivity of graphene varactor will be further discussed, demonstrating the prospective of early diagnosis as well as the potential as a sensor platform to carry different surface functionalization.

Keywords: Biosensors, Nanotechnology, Semiconductor, Sensors
Application Code: Biomedical
Methodology Code: Sensors
Current nanoparticles research in biomedical applications is heavily geared toward therapeutic applications rather than diagnostics applications. Advancement in diagnostics may lead for the ultimate therapeutic goal, personalized medicine, especially for cancer. Tumors are known to have unique chemical properties, such as low pH and low O2. Just recently, a group reported that the typical elevation of extracellular K+ concentration (5 to 10 folds increase) in the tumor microenvironment led to the suppression of the activity of immune cells. Immunotherapy of tumors has been considered as one of the most promising avenues of cancer therapy, outdoing the traditional avenues of radiation-(relevant to O2 concentrations) and chemo-therapy (relevant to pH). Despite the importance of chemical information in tumors, there are no clinically available tools for “quantitative” pH, K+, tissue O2 imaging. Photoacoustic (PA) imaging can be employed to provide chemical imaging of all target analytes for cancer (pH, O2 and K+). PA imaging is light induced and ultrasound detected. Ultrasound detection allows enhancing the penetration depth limit in vivo in comparison to fluorescence imaging (~50 folds higher). Here, methods for in vivo quantitative chemical imaging for pH, O2, and K+ are discussed.
Over the past decade, microfluidic paper-based analytical devices ([micro]PADs) have drawn significant attention as low-cost analytical platforms for point-of-care testing (POCT) or on-site analysis. However, the requirement of external optical signal readout equipment (e.g. scanner, camera) in quantification of analyte concentrations has prevented widespread application of [micro]PADs.

This work demonstrates user-friendly [micro]PADs for colorimetric determination of Ca\(^{2+}\) with equipment-free signal readout. Distance-based quantification relies on visually reading the length of a developed color, corresponding to the analyte concentration, which can be achieved without any equipment. For this purpose, ion-selective optode nanospheres (nano-optodes) are adapted to [micro]PADs for selective colorimetric detection of Ca\(^{2+}\). Nano-optodes are water-monodispersed micelle-based nanospheres containing an ion-specific ligand (ionophore) and a colorimetric pH indicator (chromoionophore), allowing for rapid optical detection of ions of interest.

For fabrication of [micro]PADs, the prepared nano-optodes as well as pretreatment reagents (pH-buffering salts and MgCl\(_2\)) were deposited on a wax-patterned paper by means of a simple thermal office inkjet printer. The number of printing cycles for the deposition of sensing reagents was evaluated. To achieve selective determination of Ca\(^{2+}\) on [micro]PADs, whole device lamination was performed to limit rapid evaporation of sample liquid during assays. Ca\(^{2+}\) assays with distance-based signal readout were successfully achieved using 30 [micro]L of aqueous CaCl\(_2\) samples, and selectivity against other cations was confirmed (e.g. Na\(^{+}\), K\(^{+}\), Mg\(^{2+}\)). The developed distance-based [micro]PADs quantified Ca\(^{2+}\) in real water samples (drinking waters and tap waters).

**Keywords:** Analysis, Clinical/Toxicology, Lab-on-a-Chip/Microfluidics, Paper/Pulp

**Application Code:** Biomedical

**Methodology Code:** Sensors
Circulating tumor cells (CTCs) have the potential as a surrogate marker in determining cancer patient’s clinical outcome and treatment response. Characterization of CTCs also offers novel insight into personalized medicine for guiding treatment decisions. However, widespread utilization of CTC assays in a clinical setting is still challenged due to the cumbersome procedure of current techniques. Here, we developed a novel optical sensing platform based on integrating bioluminescence and fluorescence techniques for the rapid detection of viable CTCs and subsequent molecular characterization of those cells at the single-cell level. Gaussia luciferases genetically fused with antibody mimetics showed the highly sensitive bioluminescence detection with high specificity against CTCs. Our platform demonstrated the capability of detecting 10 spiked tumor cells in 1mL blood, and did not cause cell toxicity. Moreover, incorporating fluorescent dyes with fusion proteins to generate dual-modality probes enabled precise identification of individual spiked tumor cells that can be isolated for single-cell analysis using current fluorescence-based single-cell isolation devices after the initial bioluminescent CTC detection. In vivo experiments using a metastatic animal model demonstrated that our platform can detect CTCs in the blood of tumor-bearing mice. Next, we demonstrated that the isolated CTCs can be grown and expanded in culture media, and individual CTCs can then be further identified and employed in downstream analysis. We combined our newly developed platform with a high-efficiency enrichment microfluidic device to make the technology amenable for clinical settings. This simple and easy-to-use platform dramatically increased the detection sensitivity for CTCs by more than 10-fold. In this presentation, we will discuss the fundamentals and potential use of our technology as a diagnostic and prognostic tool for CTC detection in clinical and point-of-care applications.
We report a portable optical cavity based biosensor system using a scaled differential detection method for use in point-of-care diagnostics. The portable system is designed to be low cost and easy to use and consists of three shelves: the optical shelf, the sample holder, and the camera. The outer case and shelves are constructed using aluminum and block ambient light from reaching inside the system. Beginning with the optics shelf, the light from two collimated laser diodes at different wavelengths are combined together through a beam splitter to allow the light to propagate along the same optical path. The lights are reflected through the sample using a mirror. The optical cavity sample is created by two partially reflecting thin film silver mirrors with a small gap to allow for a channel for fluid flow. This channel is created by fabricating a layer of SU-8 on each silver mirror, and bonding the two fabricated mirrors together using a heat based SU-8 to SU-8 bonding method. The lights reflected from the mirror propagates through the aforementioned sample and is measured by a CMOS camera. Testing will be conducted by flowing standard refractive index fluids of 1.3-1.395 (in increments of 0.005) through the channel and capturing the intensities of the two laser diodes with the CMOS camera. The laser intensities is run through the differential calculation and compared with simulation. Due to the variability in the fabrication, multiple samples with the same fabrication parameters are constructed and measured using the standard refractive index fluids to determine the variability in the measured intensity and differential value as compared with simulation.

This work was supported by NSF grant ECCS-1707049.
Rapid and accurate biosensing with low concentrations of the analytes is usually challenged by the diffusion limited reaction kinetics. Thus, long incubation times or excess amounts of the reagents are employed to ensure the reactions to go to completion. Here we propose a technology that provides electromixing of the reagents in solutions where the incubation times, or in other words, the time required for the desired molecules to meet in stationary solutions, can be reduced substantially. In this specific case, a FRET based quenching bioplatform is adopted, where a molecular beacon DNA (MB) modified with sulfhydryl (–SH) and fluorescein (FITC) dye at different terminals was incubated with 10 nm sized gold nanoparticles (AuNPs) in the wells of an integrated ELISA plate (iPlate), in which a printed circuit board (PCB) is attached at the bottom to control the electromixing. When the MB binds to AuNPs through thiolate chemistry in the solution, FITC dye comes in close proximity to the AuNP surface and the emission is quenched via FRET principle. On the other hand, the gold electrodes of the PCB also contribute to the quenching of the FITC dye on MBs so that there is dual quenching of the FITC dye. This reaction was conducted with different amounts of AuNPs to observe the kinetics of MB quenching. Total quenching efficiency of the MBs can go up to 90% while varying the AuNP quantity and these quenching efficiencies reached the plateau in about 60 minutes in DPBS buffer. When the electromixing is involved, the time required to reach the highest quenching efficiencies could be reduced by up to 4 times. Thus, it was demonstrated that this technology may improve the kinetics of the diffusion limited biological reactions substantially so that it can be adopted in various different sensing platforms for rapid measurements.
In this session, the inaugural awardee and Professor Lloyd Smith will discuss some recent advances in the field of top-down proteomics. This includes the establishment of new workflows for characterizing whole proteins and their complexes with complete molecular specificity. Kelleher also will describe the Cell-Based Human Proteome Project (CB-HPP), an effort to map the composition of 1 billion protein molecules throughout the human body for $1 each. The CB-HPP would like to invite researchers in the field of proteomics and beyond to come together to “domesticate” the human proteome, greatly advancing the field of measurement science, expanding our understanding of biological systems, and paving the way for new technologies. Of special note for a leadership award is the Consortium for Top Down Proteomics, a 501(c)3 Research Consortium established in 2012. As founding President, Kelleher helped form this Consortium in 2012 to promote innovative research, collaboration and education accelerating the comprehensive analysis of intact proteins and their complexes. This session will have contributions from Kelleher and Professor Lloyd Smith, who are both helping to grow the Consortium and top-down proteomics in general.

Keywords: Mass Spectrometry, Proteomics, Teaching/Education
Application Code: Genomics, Proteomics and Other 'Omics
Methodology Code: Mass Spectrometry
Proteoforms and Their Families – A New Paradigm for Proteomics

Proteoforms, the different forms of proteins produced from the genome with a variety of sequence variations, splice forms, and myriad post-translational modifications, are critical elements in all biological systems. Remarkably, standard “bottom-up” proteomic strategies, in which proteins are digested into peptides prior to their tandem mass spectrometric analysis, are not able to reveal the proteoforms that are present. This is because many different gene products, isoforms, and proteoforms can contain the same peptides, and thus peptide-level information alone is insufficient. The alternative strategy of “top-down” proteomics, wherein proteins are directly analyzed by tandem MS, has also faced challenges in throughput, sensitivity, and sequence coverage that have historically limited its ability to provide comprehensive information on complex proteomic samples. Tackling this interesting and important technical challenge is an exciting frontier area of modern proteomics.

Our group is developing an integrated suite of tools to enable comprehensive proteoform analysis in complex samples. Experimental data of four types is acquired: RNA-seq transcriptomic data, to elucidate the genetic variations and splice variations present; bottom-up data, to identify and quantify PTMs that are present; top-down data, to help to identify the proteoforms that are present; and intact proteoform mass data, to increase and extend the proteoforms that can be identified. We have developed the open-source software tool Proteoform Suite to integrate these data streams, construct families of proteoforms derived from the same gene, assess proteoform function using gene ontology (GO) analysis, and enable visualization of quantified proteoform families and their changes in response to perturbation. We envision this integrated and multifaceted approach to proteoform analysis as offering a new paradigm for the proteomics of complex systems.

Keywords: Bioanalytical, Bioinformatics, Mass Spectrometry, Proteomics
Application Code: Genomics, Proteomics and Other 'Omics
Methodology Code: Mass Spectrometry
DNA nanotechnology uses DNA (or nucleic acids) as a versatile material to rationally engineer tools and molecular devices at the nanoscale. During this presentation I will give an overview of the most representative and recent examples developed in our lab where we exploited the “designability” of DNA to fabricate nature-inspired DNA-based nanoswitches and nanodevices that are specifically designed to undergo a conformational change (switch) upon binding to a specific input (i.e. target). This input-triggered conformational change can be used to detect a wide range of molecular targets including antibodies, DNA sequences, pH, metal ions and transcription factors.

References

Keywords: Bioanalytical, Biomedical, Biosensors, Nanotechnology
Application Code: Bioanalytical
Methodology Code: Sensors
Natural nanomachines rely on biomolecular switches, biomolecules that undergo binding-induced changes in conformation or oligomerization to transduce chemical information into specific biochemical outputs. Inspired by these systems, we have developed several DNA-based switches and nanomachines that are activated by inputs ranging from temperature and pH, to small molecules (e.g. drugs, explosives) and large macromolecules (e.g. antibodies). These can be, for example, adapted into efficient biosensors that are selective enough to be employed directly in whole blood or into smart molecular transporters for drug delivery applications. In my talk, I will explain how we design and build these nanoswitches and show how a better understanding of natural biomolecular switches significantly helps our efforts to build switches and nanomachines with applications in medicine.

Keywords: Bioanalytical, Biosensors, Nanotechnology, Nucleic Acids
Application Code: Bioanalytical
Methodology Code: Sensors
Ingestible sensors have been hailed as the next influential tools for monitoring human health, providing invaluable information regarding chemical components of the gut. Prof Kalantar-zadeh and his group have developed a novel low-cost and non-invasive medical device called human gas sensing capsule which has applications in diagnostics of gastrointestinal disorders and for assessing dietary effects on the gut. The product is a capsule size indigestible electronic device that leaves the body after the normal bowel transient (Figure 1). The capsule consists of gas sensors, micro-electronic circuits, small-sized harmless batteries and telecommunication components. This capsule allows accurate measurement of the concentrations four vital gases of O$_2$, H$_2$, CO$_2$ and CH$_4$ and also temperature. Intestinal gas profiles are then transmitted to an external small handheld device that communicates with a smart-phone for real-time data display and analysis. Animal and the first phase of human trials have been successfully finished. The outcomes of these trials will be presented in the talk. The outcomes show some extraordinary phenomena that can potentially revolutionize fields of gastroenterology and food sciences.

**Abstract Text**

Ingestible sensors have been hailed as the next influential tools for monitoring human health, providing invaluable information regarding chemical components of the gut. Prof Kalantar-zadeh and his group have developed a novel low-cost and non-invasive medical device called human gas sensing capsule which has applications in diagnostics of gastrointestinal disorders and for assessing dietary effects on the gut. The product is a capsule size indigestible electronic device that leaves the body after the normal bowel transient (Figure 1). The capsule consists of gas sensors, micro-electronic circuits, small-sized harmless batteries and telecommunication components. This capsule allows accurate measurement of the concentrations four vital gases of O$_2$, H$_2$, CO$_2$ and CH$_4$ and also temperature. Intestinal gas profiles are then transmitted to an external small handheld device that communicates with a smart-phone for real-time data display and analysis. Animal and the first phase of human trials have been successfully finished. The outcomes of these trials will be presented in the talk. The outcomes show some extraordinary phenomena that can potentially revolutionize fields of gastroenterology and food sciences.

**Keywords:** Biosensors, Gas, Medical

**Application Code:** Clinical/Toxicology

**Methodology Code:** Sensors
Advances in Measurement Science Lectureship Awards

Hydrogen Doped Plasmonics of 2D Molybdenum Oxides for Creating Ultra-Sensitive and Fast Kinetics Biosensors

The unique dopant dependent plasmonics of degenerately doped semiconductors can offer new dimensions in biosensing, especially enabling efficient detection of certain biochemical events which cannot be achieved by the conventional refractive-index mediated plasmonic sensors. However so far, operation wavelengths of degenerately doped semiconductors are incompatible with most of current optical systems. Substoichiometric plasmonic compounds as alternatives can blue-shift the resonance wavelength to near infrared (NIR) but are offset with reduced sensitivity and kinetics. In this work, such a challenge is addressed through the demonstration of degenerately hydrogen doped plasmonics of two-dimensional molybdenum oxides in a representative enzymatic glucose sensing model. The plasmon resonance wavelength can be tuned across both visible and NIR region, and the intercalated H\(^+\) are easily released from the host structure during enzymatic oxidation of glucose (Figure 1a). The plasmonic response ratio and linearity are influenced by the H\(^+\) content and intercalation location, in which the optimum response is found at \(x=1.55\) for hydrogen intercalated molybdenum oxides \((H_{1.55}MoO_3)\). This novel plasmonic biosensing platform can be readily adapted to a simple and low-cost LED-photodetector setup (Figure 1b), achieving a detection limit of 2 nM at 410 nm with a response time less than 10 s (Figure 1c-e). The performance is superior over most of the current enzymatic glucose sensors, providing a real commercial opportunity in developing high performance tunable plasmonic biosensors.

Keywords: Bioanalytical, Biosensors, Biotechnology, Sensors
Application Code: Bioanalytical
Methodology Code: Sensors
New tools have the potential to unlock unexpected insights into biology. Our group extending the toolbox for cellular imaging by developing an array of nanosensors for the measurement of ion and small molecule concentrations in vitro and in vivo. Each sensor is based on a polymeric platform and a modular format. Our sensors are easily tuned for dynamic range and extendable to new analytes, such as sodium, histamine, glucose, and neurotransmitters. Previously, we developed a nanosensor the reported acetylcholine levels via fluorescence. Although the sensor was ratiometric and emitted in the visible wavelength range, the depth that these sensors could be placed in tissue was limited by the use of fluorescence. Specifically, our studies demonstrated the use of the sensors for imaging in neural tissue slices, but would not be extendable to whole-brain imaging due to issues with scatter and absorbance of the signal. We overcame the depth limitations by substituting a MR reporter for the fluorescent indicator on the nansosensor. The analytical characteristics, including dynamic range, sensitivity, and selectivity will be discussed. In addition, we will demonstrate the use of the sensor for dynamically tracking acetylcholine release in the prefrontal cortex upon drug stimulation. Finally, we will consider the inherent trade-offs between imaging methods. Ultimately, by combining advanced imaging techniques with our array of nanosensors, we plan to gain a better understanding chemical dynamics at a greater depth in vivo.
For quantitative measurements in biological systems, luciferases have the advantage of providing highly sensitive detection with linearity extending over several logs. Adapting these capabilities into biosensor designs requires that conditional constraints be incorporated into the bioluminescent mechanism. The NanoLuc luciferase may be particularly suited for structural engineering due to its small size (19kDa) and single-domain structure. We have demonstrated that this enzyme can be divided into two complementary domains, whose affinity can be varied 100,000 fold through amino acid substitutions. Conditional modulation of domain interactions can serve as the foundation for detecting molecules or biomolecular events.
FRET-sensors have become important tools for intracellular imaging, but their dependence on external illumination can result in photobleaching and phototoxicity, which limit measurements over extended periods of time. Fluorescence measurements also suffer from autofluorescence and light scattering, which hampers in vivo imaging and measurements in strongly absorbing and scattering media such as blood. In principle, these issues can be resolved by using sensors based on bioluminescence resonance energy transfer (BRET). The recent development of brighter and more stable luciferases and the concomitant improvement in luciferase substrates have substantially decreased the sensitivity gap between fluorescence and bioluminescence. As a result, BRET has emerged as an attractive sensor format for point-of-care diagnostics and intracellular imaging in single living cells. In my presentation I’ll show how modular protein sensor design strategies originally developed for FRET sensor proteins are easily adapted for the development of robust BRET and dual BRET/FRET sensors. In particular I’ll focus on the development of various BRET-based homogenous assays that allow detection of antibodies, small molecules, metal ions and DNA/RNA directly in complex matrices such as blood plasma with minimal sample handling. LUMABS (LUMinescent AntiBody Sensors) will be introduced as a generic platform for the direct detection of antibodies in solution. In addition to the direct application of LUMABS sensors for monitoring of therapeutic antibodies, I’ll also present our work on alternative BRET-based homogenous immune assays and the further development towards point-of-care diagnostic assays.

**Keywords:** Bioanalytical, Biosensors, Immunoassay, Luminescence

**Application Code:** Bioanalytical

**Methodology Code:** Fluorescence/Luminescence
The mitochondrion is the powerhouse of the cell and also plays a key role in regulating programmed cell death. In particular, the elucidation of the mitochondrion’s function in various human diseases has generated an appreciable amount of interest in exploring this organelle as a potential drug target. Delivering molecular agents to mitochondria is challenging, however, due to the difficulty of penetrating the hydrophobic inner mitochondrial membrane. We developed a set of peptide-based agents that are able to carry drug molecules into mitochondria, and have studied the effects of delivering anticancer and antibacterial agents to mitochondrial targets. We have also mitochondrially-targeted drug conjugates to discover new proteins with previously undetected activities in this cellular subcompartment. Recently, we generated new mitochondria-specific probes that are able to monitor simultaneously local polarity and viscosity in this cellular microenvironment. This presentation will focus on this new class of probes and their application to the study of mitochondrial biology.
Hollow, or porous nanomaterials are attractive as drug delivery vehicles because they can provide the advantages of sustained release, lower systemic toxicity, and tissue-specific targeting while protecting the drug payload from degradation. As an inorganic material, porous silicon (pSi) has been exploited as a drug carrier because of its biocompatibility, its tunable pore structure, and its high capacity to carry therapeutic molecules. Theranostic systems that are self-reporting, such that they indicate the status of the delivery vehicle in terms of local drug concentration or residual drug capacity, fill a need to monitor the drug delivery process. This is particularly important for long-acting formulations such as in the treatment of diseases of the eye or in the delivery of hormonal contraceptives. Theranostic nanomaterials are poorly developed at present, but they offer substantial benefits in personalized medicine by avoiding use of indirect methods of drug quantification such as sampling blood or urine. A unique dimension of pSi is that it contains intrinsic photoluminescent domains in its nanostructure. It is one of few semiconductor "quantum dot" materials that is non-toxic and that degrades to non-toxic byproducts. For self-reporting drug delivery applications, advantages of pSi include: (1) the photoluminescence appears at tissue-penetrating near-infrared wavelengths; and (2) the long-lived (microseconds) excited state of pSi enables elimination of interfering background fluorescence by time-gated imaging. This presentation will discuss the use of photoluminescence to self-report drug delivery in vivo.

Keywords: Biomedical, Biosensors, Biotechnology, Nanotechnology
Application Code: Nanotechnology
Methodology Code: Sensors
Assessing in situ nanoparticles (NPs) internalization at the level of a single cell is a difficult but critical task in the context of efficient nanomedicine perspectives. One of the main actual challenges is to control the number of internalized NPs per cell. To in situ detect, track, and above all quantify NPs in a single cell, we propose an approach based on a multimodal correlative microscopy (MCM), via the complementarity of three imaging techniques: fluorescence microscopy (FM), scanning electron microscopy (SEM), and ion beam analysis (IBA). MCM was performed on single targeted individual cell cultured and maintained on a specifically designed sample holder, to probe either dye-modified or bare NPs.

The data obtained by both FM and IBA on dye-modified NPs were strongly correlated in terms of detection, tracking and colocalization of fluorescence and metal detection. IBA techniques should therefore open a new field concerning specific studies on bare NPs their toxicological impact on cells. Complementarity of SEM and IBA analyses provides surface (SEM) and in depth (IBA) information on the cell morphology as well as on the exact localization of the NPs. Finally, IBA also provides in situ quantification of all the chemical elements present in this single cell including the exogenous ones (NPs). This unique feature opens further insights in dose-dependent response analyses and adds the perspective of a better understanding of NPs behavior in biological specimens for toxicology or nanomedicine purposes.
In recent years, single particle inductively coupled plasma mass spectrometry (spICPMS) has emerged as a reliable tool that can both count metal-containing nanoparticles (NPs) and measure their mass, thereby allowing sizing if their shape, density and composition are known. However, the methodology associated with the current spICPMS approach for mass determination requires determination of both the sample uptake rate and the sample introduction efficiency of the nebulization system. The use of a sequential mass spectrometer limits the number of elements that can be measured in NPs to one. A fast data acquisition system is also required, as any significant settling time, including during the measurement of a single mass-to-charge ratio, leads to over-estimation of particle size. During this presentation, different approaches that can circumvent these limitations will be presented. This includes flow injection (FI) analysis coupled to ICPMS, i.e. FI-spICPMS, with which the determination of the transport efficiency and of the sample uptake rate is not required for the accurate measurement of particle mass. It also only requires a measurement of the transport efficiency for determination of the particle number. Furthermore, because of the dilution that is inherent to the FI process, the precision associated with particle mass and size measurements is improved compared to the conventional spICPMS approach. A simple correction for settling time will be described, which allows accurate particle size measurement when the settling time is significantly larger than the dwell time. A similar approach should also enable the measurement of more than one element per NP using sequential mass spectrometers. Each approach will be demonstrated using selected examples of application.
Heterogeneity of subcellular compartments (organelles) is essential to cell function. Perturbation of such heterogeneity is associated with multiple diseases and aging. Traditionally, studies linking subcellular properties with normal cell function, disease, and aging rely on preparative bulk methods (e.g. mechanical disruption and centrifugation) to isolate and characterize subcellular properties of isolated organelles. These methods hide the heterogeneity displayed by the isolated organelles and is impeding progress on prioritizing molecular targets of treatment. This presentation describes our ongoing efforts to develop methodologies to analyze individual isolated organelles and compares such methodologies.

We will present the use of particle tracking and capillary cytometry to characterize lipid droplets (organelles that store lipids and sterols in cells), how we have addressed complications due to their lipophilic nature, and how these technologies complement each other. Our findings will include the description of size distribution of lipid droplets isolated from young and geriatric mice. Ongoing work to expand these methodologies to other organelle types and their properties will be discussed.

Frequently, individual organelle analysis requires simultaneous detection of multiple organelle types, which cannot be easily distinguished with conventional particle tracking. We will present the use of mass cytometry to detect coexisting types of individual organelles. Our strategy uses lanthanide-tagged antibodies detect individual organelles. For the first time, this presentation will demonstrate feasibility of using this technology on the analysis of organelles, opening possibilities to understand subcellular heterogeneity of each organelle type in mixtures of different organelle types.

Keywords: Biotechnology, Fluorescence, ICP, Particle Size and Distribution
Application Code: Bioanalytical
Methodology Code: New Method
Ratchets are known for their intriguing properties of transporting particles even if no net force is applied. Even more stunning for separation purposes is the fact that ratchet migration may occur simultaneously with normal migration for particles in a particular mixture. This opens the possibility to transport particles with different properties at different velocities and – in some cases – even into different directions. Ratchet migration mechanisms have been shown for microparticles in various realizations, but applications to bioparticle separations have been limited. Here, we present how ratchet migration and a related migration mechanism termed absolute negative mobility can be exploited for the separation of sub-µm based nanoparticles and subcellular organelles. The two approaches are based on tailored microfluidic devices with unique geometrical features inducing the non-intuitive migration mechanisms. In order to improve the speed of ratchet based separations and the selectivity for particles down to the sub-µm regime, we combine underlying electrical driving effects with dielectrophoresis in microfluidic devices. To find suitable driving mechanisms and optimize particle separation we developed a numerical model based on finite element software tracking thousands of particles simultaneously and allowing to extract statistical separation parameters. We further demonstrate ratchet migration with sub-µm sized polystyrene beads as well as liposomes as model biological particles. Moreover, we demonstrate the potential of ratchet based migration mechanisms for mitochondria, capable of steering differently sized organelles into different directions. This can be exploited for size-based separation of mitochondria and is important to access mitochondria sub-populations, which have been linked to a variety of malfunctions in cellular processes.

Keywords: Bioanalytical, Electrophoresis, Lab-on-a-Chip/Microfluidics, Separation Sciences
Application Code: Bioanalytical
Methodology Code: Microfluidics/Lab-on-a-Chip
We have developed microfluidic and nanofluidic systems for the study of single nanoparticles, ranging from individual synaptic vesicles and exosomes to single semiconducting polymer dots. Here, we will describe our technical approach, device performance, and the new information we learned about these nanoparticle systems as revealed by the new measurements.
P-polarized multiple-angle incidence resolution spectrometry (pMAIRS) is a cutting-edge spectroscopic technique, which enables us to analyze the molecular structure in a thin film through quantitative molecular orientation analysis. pMAIRS is robust to surface roughness of the thin film, and therefore various kinds of thin films prepared by using the spin-coating, drop-casting, dip-coating and bar-coating techniques can be analyzed with a high reproducibility and a high accuracy of molecular orientation. Since the quantitative analysis requires no optical parameters such as film thickness and refractive index, pMAIRS opens up doors to various chemical applications, which needs surface and thin-film analyses.

In this talk, some representative applications in both industries and academia are presented. Since the pMAIRS equipment has been released very recently in North America, some enthusiastic applications mostly performed in Japan will be reported, which covers analyses of polymer reaction in a thin film, molecular adsorption on nano-wires, molecular arrangement in a solar cell and surface coating of detergent.

Keywords: FTIR, Material Science, Membrane, Surface Analysis
Application Code: Material Science
Methodology Code: Vibrational Spectroscopy
Characterising in situ the chemical composition, size and morphology of aerosol particles is crucial for understanding the role of aerosol in a broad range of disciplines from the atmosphere, to drug delivery to the lungs and to combustion. Raman spectroscopy has been used widely over many decades to characterise the composition of aerosol particles. In this talk we will explore the use of both linear and non-linear Raman spectroscopy in the analysis of the properties of individual particles spanning from 1 to 100 \( \mu \text{m} \) in diameter over timescales spanning from 1 ms to days captured in optical or electrodynamic traps. Liquid droplets in this size range act as optical cavities, facilitating the enhancement of Raman scattering at wavelengths that match whispering gallery modes (WGMs). The measured wavelengths of WGMs can be compared with Mie scattering calculations to determine the radius and refractive index (RI) of droplets with an uncertainty of <0.02 %. Such highly refined measurements of size and RI can provide a method for non-intrusively probing the dynamics of processes than transform the physicochemical properties of the aerosol. In particular, we will see how processes such as the partitioning of volatile species between the gas and condensed phases, chemical reactions, and particle interactions and coalescence can be interrogated using Raman spectroscopy.
Challenges in Surface Vibrational Spectroscopy

A Highly Sensitive, Selective and Reproducible 3D SERS Sensor for Detection of Toxins in the Environment

Approximately 1 billion pounds of pesticide and 44 billion pounds of fertilizers are used every year in the U. S., and the latter often leads to algal blooms and fish kills when washed into open and contained waters. Development of an early-warning sensing platform that is sensitive, selective and reproducible is a challenge that scientists must confront. To this end we have developed a 3-D, nanocomposite surface enhanced Raman scattering (SERS) substrate composed of a homogeneous and highly dense silver coated gold nanorod (Ag/AuNR) assembly on electrospun polycaprolactone (PCL) microfibers using electrostatic interactions as the driving force. Specifically, positive charges on the PCL fibrous substrate were established via polyelectrolyte layer-by-layer (LBL) deposition of poly(diallyldimethylammonium chloride) (PDADMAC) and poly(sodium 4-styrenesulfonate) (PSS) with the former being the outermost positively charged layer. Decoration of a PSS layer onto the Ag/AuNRs imposed negative charges on the nanorod surface. Driven by the attractive electrostatic interaction, immobilization of the Ag/AuNRs on the PCL fibers was initiated upon substrate immersion and the kinetics of the immobilization process were investigated. SEM characterization of the Ag/AuNR/PCL nanocomposite fibers revealed a uniform nanorod coating on the fiber surface with the immobilized Ag/AuNR density being high enough to provide full surface coverage. This substrate allows detection of 4-methylpyridine (4-Mpy) at a concentration as low as 10⁻⁸ M (10 nanomolar) with excellent reproducibility. SERS substrates fabricated with Ag/AuNRs with different Ag coating thicknesses have been compared with substrates using AuNRs. It was found that when fabricated with a Ag coating, the substrates exhibited larger SERS enhancement than that found for substrates fabricated with AuNRs. This could be attributed to electron transfer between the gold core and silver shell in a bimetallic rod-shape nanostructure.

Keywords: Materials Characterization, Surface Enhanced Raman Spectroscopy, Trace Analysis, Vibrational Spectroscopy
Application Code: Material Science
Methodology Code: Sensors
Challenges in Surface Vibrational Spectroscopy

Structured Illumination Compressive Hyperspectral Sum Frequency Generation Microscopy

Sum frequency generation spectroscopy (SFG) is a useful technique to study molecular properties of surfaces. As a second-order technique it is uniquely sensitive to the average organization of molecules at the surface. However, as most surfaces are spatially heterogeneous, it is difficult to interpret the spectrum as single domain. The development of SFG into a microscopy has allow a more detailed and accurate analysis of the spatio-spectro-temporal evolution of the surface chemistry. The SFG microscope development will be presented, as well as the use of compressive sensing and the application toward corrosion inhibition and self-assembled monolayers.

Keywords: Imaging, Microscopy, Surface Analysis, Vibrational Spectroscopy
Application Code: General Interest
Methodology Code: Surface Analysis/Imaging
Raman spectroscopy has a reputation for being an insensitive technique for surface analysis, with scattering cross-sections about 13 orders of magnitude lower than fluorescence and ten orders lower than absorption cross-sections in IR spectroscopy. It also lacks the intrinsic surface sensitivity of sum-frequency spectroscopy. Raman spectroscopy does have advantages over IR in applications that involve wet interfaces (water is a weak Raman scatterer but absorbs strongly across much of the mid-IR), high spatial resolution (the diffraction limit is determined by wavelengths in the visible rather than the mid-IR) or long wavelengths (where lab-based IR sources are weak and detectors insensitive). The lack of surface sensitivity of linear spectroscopic techniques can be militated by the use of evanescent waves for IR absorption (ATR-IR) or Raman scattering (TIR-Raman). Working near the critical angle enhances the electric fields at the surface and collection of the scattered light through the substrate increases the efficiency. With careful optimisation, it is possible to acquire high-quality Raman spectra from nm-thick films at solid-water interfaces in a few tens of seconds and, with the aid of chemometrics, to acquire kinetic information with a time resolution of a second – without the use of surface plasmon enhancement. Recently, we have shown that nm-thick films can also be imaged with 1-micron resolution by use of a tuneable filter. Currently, we are building a Hadamard transform Raman spectrometer to yield full hyperspectral data sets with diffraction-limited resolution and an acquisition time orders of magnitude faster than a conventional point mapping approach. This talk will set out the challenges in surface Raman spectroscopy, illustrate the performance that can be achieved now and suggest ways that some of the remaining challenges may be overcome.
### Disease Diagnosis Enabled by Integrated Microfluidic Systems

#### Solution Microarrays for Cross-Reaction Free Multiplex Immunoassays

This talk will describe a cost-effective, user-friendly, and anti-body cross-reaction free multiplex immunoassay that works in a conventional 96 well plate format. The method uses aqueous reagent droplets arrayed within an immiscible sample aqueous solution. Pre-arrayed and dried reagent spots rehydrate spontaneously upon sample solution addition to enable convenient sample processing with fewer wash and reagent addition steps. Stable partitioning and localization of antibodies within the droplets enable use of orders of magnitude less detection antibodies and also eliminate unwanted antibody cross-reactions. The ability to stably partition antibodies and other reagents enable spatial multiplexing even of homogeneous immunoassays where all reagents are diffusing freely in solution. Some applications include detection of protein biomarkers from graft versus host disease patients as well as from in vitro cell cultures.

**Keywords:** Immunoassay

**Application Code:** Biomedical

**Methodology Code:** Fluorescence/Luminescence

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<td>Primary Author</td>
<td>Shuichi Takayama</td>
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<td>Co-Author(s)</td>
<td>Georgia Tech</td>
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Since their inception over 25 years ago, microfluidic devices have been envisioned for their potential to facilitate disease diagnosis. Here, we present progress in developing microfluidic systems for two distinct medical conditions: sepsis and preterm birth.

We are developing a microfluidic platform for the rapid determination of bacterial blood infections. Our efforts have focused on selective extraction of target nucleic acids from bacterial lysate. We have demonstrated selective capture of specific DNA sequences on a monolith in a microfluidic channel, and elution of the DNA for subsequent detection [1]. We have obtained recoveries >80% for target DNA, while mismatched sequences are captured with 50-fold reduced efficiency compared to target [1]. We are now working on combining our DNA capture module with bacterial purification from blood and single-molecule detection to create a complete analysis system.

We are also developing integrated microfluidic devices that analyze for protein and peptide biomarkers from maternal serum that predict risk for preterm birth (PTB). We have developed microchip electrophoresis conditions for 7 of these biomarkers. We have also created integrated microfluidic devices that combine solid-phase extraction, on-chip fluorescent labeling and electrophoretic separation of PTB biomarkers [2]. We are presently developing 3D printing methods that create truly microfluidic features [3,4] for complex, integrated devices that would be difficult or even impossible to create with conventional microfabrication. Our microfabrication methods and integrated devices offer excellent capabilities for advancing the rapid diagnosis of diseases.

We thank the NIH (R01 EB006124 and AI116989) for financial support.

References

Keywords: Automation, Capillary Electrophoresis, Lab-on-a-Chip/Microfluidics, Solid Phase Extraction
Application Code: Bioanalytical
Methodology Code: Microfluidics/Lab-on-a-Chip
Flow-gated capillary electrophoresis (CE) employs a silica capillary for separations while taking a cross configuration for rapid sample injection, and thus flow-gated CE can be regarded as the hybrid of conventional and microchip CE. Flow-gated CE has numerous advantages over microchip CE: the separation capillary can use various inside diameters as small as 5 µm; a clogged or damaged capillary can be easily replaced with a new one which is inexpensive; the capillary is more compatible with popular detectors such as mass spectrometry and laser-induced fluorescence detection using a sheath-flow cuvette or an oil-immersing objective; high separation efficiency can be achieved in the circular capillary channels; and sample processing steps can be conveniently integrated with a flow-gated CE system. This presentation will demonstrate the capability of flow-gated CE in quantitative analysis of biomarkers in urine samples. First, rapid determination of free prolyl dipeptides, especially prolyl-hydroxyproline, in urine samples was performed by using cholate and deoxycholate in MEKC, and the separation was obtained in 30 s, which was enabled by the high separation efficiency with a 10-µm capillary. Second, 20 amino acids in urine samples were separated in 90 s by using MEKC and were quantified by coupling alternate injections of a sample and its standard additions. Third, dopamine and norepinephrine in cerebrospinal fluid were pre-concentrated and quantified in flow-gated CE by employing borate complexation with catecholamines after fluorogenic derivatization by naphthalene-2,3-dicarboxaldehyde. The selective online enrichment strategy achieved over 100-fold detection enhancement, which is valuable to detect catecholamines in various biological samples. In addition, microdialysis was often coupled with on-line derivatization; and a micro-switch was coupled with flow-gated CE for alternate injections to compensate for the drift of the detection system.

**Keywords:** Bioanalytical, Capillary Electrophoresis, Method Development, Separation Sciences

**Application Code:** Biomedical

**Methodology Code:** Microfluidics/Lab-on-a-Chip
**Session Title**: Disease Diagnosis Enabled by Integrated Microfluidic Systems  
**Abstract Title**: Integrated Microfluidic Systems for the Investigation of Oxidative Stress  
**Primary Author**: Susan M. Lunte  
**Author**: University of Kansas  
**Co-Author(s)**: Christopher T. Culbertson  

**Abstract Text**  
Microchip electrophoresis is a powerful tool for the analysis of biological samples. In particular, its ability to perform fast, efficient separations makes it possible to monitor several compounds simultaneously with high temporal resolution. The small dimensions of the channels in the chip are compatible with the analysis of microdialysis samples and single cells. Application of microchip electrophoresis (ME) with both electrochemical and fluorescence detection for the determination of reactive nitrogen and oxygen species (RNOS) in immune cells will be presented. This includes direct amperometric detection of RNOS as well as the evaluation of fluorescent reagents used for specific chemical species. ME allows detection of multiple substances in a single run, giving a better snapshot of the total RNOS production in the cells. Progress toward the goal of high throughput single cell analysis of RNOS using microchip electrophoresis will also be presented.

**Keywords**: Bioanalytical, Lab-on-a-Chip/Microfluidics  
**Application Code**: Clinical/Toxicology  
**Methodology Code**: Microfluidics/Lab-on-a-Chip
Diabetes is now recognised as one of the fastest growing chronic conditions globally, with an estimated 422 million people affected in 2014. While diagnosis and effective treatment can reduce impacts from complications such as impaired eyesight, cardiac failure and stroke, it is estimated that for every diagnosed case there is another which remains undiagnosed. Point of care testing (POCT) devices have been available for over 2 decades for blood glucose measurements, but it is now recognised that more effective pre-diabetic monitoring and diagnosis of diabetes is achieved through including determination of the ratio of glycated haemoglobin (HbA1c) from total haemoglobin (Hb), providing a medium-term measure (8-12 weeks) of average blood glucose levels. While a number of platforms exist for POCT for HbA1c, these devices generally do not provide an intuitive collection process and are not designed to be used by the end user without supervision.

This presentation will introduce a new microfluidic approach for spectroscopic ratiometric measurement of HbA1c from Hb that can be combined with an amperometric fasting glucose sensor. Integration of the analysis with a user-friendly blood microsampling device will also be described. The determination of HbA1c ratio from dried blood spots will also be presented.

Keywords: Bioanalytical, Capillary LC, Protein, UV-VIS Absorbance/Luminescence
Application Code: Bioanalytical
Methodology Code: Microfluidics/Lab-on-a-Chip
Enhancing the safety of fresh and minimally processed produce remains a critical challenge to our agriculture and food systems. The food and agriculture industries would therefore benefit greatly from rapid sensing mechanisms to determine the presence of potential pathogens in complex matrices. Indeed, reliable, cost-effective early detection of pathogens can help reduce foodborne illness by giving farmers, distributors, manufacturers and retailers rapid knowledge about the presence or absence of pathogens and other contaminants in their production system. Advances in nanotechnology and synthetic biology, and in particular our new ability to design custom hybrid bio-inorganic nanomaterials, have given us new tools in addressing these grand challenges to the safety and security of our food supply. Until recently, the concept of “nanobots” has been relegated to science fiction, yet in proof of principle research, we have demonstrated the ability to synthesize a hybrid bio-inorganic system consisting of engineered viruses which have been magnetized using oriented conjugation to magnetic nanoparticles. While nanotechnology has enabled the synthesis of high-speed magnetic particles, synthetic biology allows us to “program” the virus to perform customized functions. Magnetized, engineered phage ‘nanobots’ can recognize and separate bacteria while infecting them, thus forcing the expression & release of reporter enzymes. Efficacy in separation and concentration from authentic agricultural samples and detection with high selectivity and sensitivity will be demonstrated. The magnetized phages were engineered carry custom reporter enzymes allowing ultra-sensitivity using several transduction formats including colorimetric, electrochemical, and bioluminescence.

The ability of genetically engineered phages to rapidly separate, concentrate, and detect bacteria will allow significant benefits to several fields including food safety, environmental monitoring, and medicine.

Keywords: Bioanalytical, Biosensors, Food Safety, Genetic Engineering
Application Code: Food Safety
Methodology Code: Portable Instruments
A facile synthetic approach to prepared dispersed, lipid protected gold nanoparticles was demonstrated herein. Such nanoparticles were capable of resisting aggregation in high-salt or detergent-containing solutions, and can be further engaged in the development of a novel sensor for monitoring peanut agglutinin (PNA). The feasibility of such sensor in studying the thermodynamic behaviors of the interaction between peanut agglutinin and ganglioside (GM) on lipid membrane is confirmed. 3-D configuration of the lipid protected gold nanoparticles enables the reinforcement of the multivalent binding effect of GM receptor to PNA ligand comparing to that in the planner lipid bilayer format. The effect of cholesterol is found to enhance affinity of GM toward PNA molecules significantly, which should be attributed to clustering of lipid rafts. Furthermore, results obtained by atomic force microscopy (AFM) confirmed the positive correlation between the cholesterol level and the size of lipid rafts.

Keywords: Analysis, Atomic Force Microscopy (AFM), Bioanalytical, Lipids
Application Code: Bioanalytical
Methodology Code: New Method
Point-of-care tests have been championed as potential game changers in the fields of human and veterinary medicine for the past few decades, however, few have succeeded in truly reaching the point-of-care or having the desired health impacts. This talk will discuss technical and market barriers to developing and implementing point-of-care diagnostic tests especially as they pertain to resource-limited settings.

Keywords: Bioanalytical, Clinical Chemistry, Clinical/Toxicology, Food Safety
Application Code: Bioanalytical
Methodology Code: Integrated Sensor Systems
Rapid and quantitative in-field testing of food and beverages for bacteria is of growing interest for government, farmers, industrials and even end-consumers to detect and prevent pathogenic outbreaks. Light-weight and globally available, paper is a good candidate for portable, low-cost, simple, microfluidic devices that permit efficient and convenient in-field analyses. Although colorimetry is frequently used for paper devices, we focused on electrochemical devices. Electroanalytical methods permit rapid quantification over wide dynamic ranges, with low limits of detection, and without interferences from color, dust or particles in samples. We added stencil-painted electrodes onto paper-based devices previously designed for bacterial culture. The presence of bacteria on the electrodes, affects the electrochemical characteristics of the system. Those changes, measured by electrochemical impedance spectroscopy, correlate to the bacterial count. Immobilizing specific capture antibodies in the vicinity of the electrode could permit selectivity to a particular bacteria.

Using paper-based devices has multiple advantages such as easy manufacturing, sample collection and disposal (burning); and if the sample counts only few bacteria, the device can be incubated to allow for bacterial growth and tested again without additional handling of the sample.

In some cases, however, a rapid quantification of bacteria at levels less than 10 cfu/mL is critical and waiting on incubation or other amplification steps would be detrimental. We are also developing another detection method based on electrochemically generated luminescence (ECL). This reverse assay relies on bacteria disturbing ECL. In absence of bacteria ECL is generated, but upon addition of bacteria the ECL decreases proportionally to the amount of bacteria present. By implementing the detection mechanism in elastomeric microfluidic devices, we aim to answer the challenge of detecting single bacterium without culture.

Keywords: Bioanalytical, Electrochemistry, Environmental/Biological Samples, Food Safety
Application Code: Bioanalytical
Methodology Code: Electrochemistry
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.

We develop a range of nanomaterials focusing on their analytical function. Liposomes for example are designed as a highly flexible signal amplification strategy for bioanalytical sensors. Taking advantage of their large inner cavity for the entrapment of marker molecules, as well as their phospholipid bilayer for modifications with biorecognition elements, we can lower limits of detection in comparison to other amplification strategies such as enzymes and nanoparticles.

Electrospun nanofibers (see figure showing a model a non-woven nanofiber mat) provide an immense potential to enhance bioassays. The great variety of chemical surfaces available and the large surface-to-volume ratio promise to solve challenges of signal enhancement, non-specific binding and analyte pre-concentration. Our research demonstrates successful development of conductive nanofibers for sensitive and sophisticated electrochemical assays, optical nanofibers with entrapped luminescent nanoparticles for highly localized detection in tissue, and nanofibers function as separation material in microfluidic channels.

Keywords: Bioanalytical, Biosensors, Food Safety, Nanotechnology
Application Code: Food Safety
Methodology Code: Microfluidics/Lab-on-a-Chip
The multiplexed detection of protein and microRNA biomarkers with microarrays has exploded onto the biosensor scene over the past decade. Label-free multiplexed detection methods such as surface plasmon resonance imaging (SPRI) and other spectroscopic and electrical techniques have achieved sufficient sensitivity (picomolar or femtomolar concentrations in microliter volumes (1)); however, all of these methods typically require the fabrication of high fidelity microarrays of purified proteins or antibodies. Our research group has been developing on-chip fabrication strategies for the creation of protein microarrays that utilize a combination of templated biosynthesis (cell-free in vitro translation and transcription, IVTT) and subsequent self-assembly of the protein microarray on the SPRI chip for immediate use in SPRI surface bioaffinity measurements.(2,3) In this talk we will describe our use of Zinc finger fusion protein tags as a method for self-assembling protein microarrays and also the inclusion of rapid nanoparticle-based capture methods for the ultrasensitive SPRI biosensing of protein biomarkers using these microarrays.


The past decades have witnessed dramatic expansion and increasing adoption of label-free optical biosensors for drug discovery. This presentation will discuss how to apply these biosensors in various stages of early drug discovery process with a special focus on high-throughput phenotypic screening. Discovery of novel GPR35 ligands and a novel linkage between G protein-coupled receptor signaling and purine de novo biosynthesis will be used to showcase the power of label-free optical biosensors for drug screening.
Label-free and high throughput tools to interrogate chemical and biomolecular interactions are desirable for many (bio)chemical applications, including therapeutic target discovery and drug development. We are exploring applications of silicon photonic sensor arrays for label-free detection of biomolecular interactions. Moderate throughput and minimal reagent consumption are achieved through spatial multiplexing, which allows multiple interactions to be probed simultaneously and within the same reaction volume. This presentation will focus largely on applications for probing biomolecular interactions important in regulating blood coagulation, which presents an additional challenge due to the requirement that these protein-lipid and protein-protein must be interrogated at model cell membrane interfaces. Recent progress in this area using both microring resonators and supporting microfluidic tools for low input biomolecular screening applications will be presented.

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

**Application Code:** Bioanalytical

**Methodology Code:** Microfluidics/Lab-on-a-Chip
Label-Free Methods for High-Throughput Screening

High Throughput Ion Mobility-Mass Spectrometry Metabolomics for Prostate Cancer Detection

Current screening approaches for prostate cancer (PCa) include the prostate-specific antigen (PSA) blood test and digital rectal examination. However, the PSA test suffers from over-diagnosis and overtreatment, and digital rectal exams are limited by low detection rates in non-palpable PCa growths. Metabolomics is being increasingly applied to explore disease biomarkers, with mass spectrometry (MS)-based platforms being widely used due their intrinsic sensitivity. The majority of metabolomics studies employ chromatography or capillary electrophoresis prior to MS analysis, which are relatively time consuming. Here, we show the high-throughput metabolic profiling of serum samples from PCa patients and healthy subjects using flow injection (FI) electrospray traveling wave ion mobility spectrometry (TWIMS) time-of-flight (ToF) MS. Compared with traditional techniques, the FI-TWIMS-ToF-MS method offers distinct advantages of rapid sample analysis and separation of compounds based on their differences in collision cross section — compounds with different charge states and classes fall into distinct regions in the m/z vs. drift time plot, facilitating metabolite structural identification with reduced false-positive assignments. In this study, a panel of 10 chemically-identified compounds predicted the presence of PCa in serum samples with 90.2% sensitivity, 83.3% specificity and 87.4% accuracy, using orthogonal partial least squares discriminant analysis, showing the feasibility of detecting PCa by means of high throughput TWIMS-MS.

Keywords: Mass Spectrometry, Metabolomics, Metabonomics

Application Code: Bioanalytical
Methodology Code: Mass Spectrometry
Protein glycosylation, the post-translational attachment of complex oligosaccharides (glycans), is the most abundant polypeptide modification in eukaryotes, and is integrally involved in human biology and disease. Changes in glycosylation have also been shown to modulate the pharmacokinetics and potency of protein therapeutics and vaccines, making them critical quality attributes for biotechnology applications. However, the study of glycans in native systems (glycobiology) and the intentional manipulation of protein glycosylation patterns (glycoengineering) remain limited by a shortage of methods to characterize glycosylation enzyme activities and a reliance on endogenous glycosylation systems which can produce highly variable glycosylation patterns and constrain choices of production hosts, protein trafficking, and possible glycoforms. Here we describe an in vitro platform for high-throughput expression and characterization of glycosylation enzymes using Escherichia coli Cell-Free Protein Synthesis (CFPS) and Self-Assembled Monolayers for Desorption Ionization Mass Spectrometry (SAMDI-MS). This workflow allowed us to produce >800 µg/mL of a cytoplasmic N-linked glycosyltransferase (NGT) in vitro and determine its peptide and sugar acceptor specificities at unprecedented depth and throughput with >2,600 unique peptide substrates. We used this dataset to develop a small, robust acceptor sequence motif (GlycTag) to direct the efficient installation of N-linked glycans onto the internal loops of heterologous protein substrates in vitro and in the cytoplasm of living E. coli. Notably, this N-linked glycosylation system does not require protein transport across cellular membranes or the use of membrane bound components.
We investigate the solution and fibril conformations and structural transitions of the polyglutamine (polyQ) peptide, D[sup]2[/sup]Q[sup]10[/sup]K[sup]2[/sup](Q10), by synergistically using UV Resonance Raman (UVRR) spectroscopy and Molecular Dynamics (MD) simulations. We show that Q10 adopts two distinct, monomeric solution conformational states, a collapsed [beta]-strand and a PPII-like structure that do not readily interconvert. This clearly indicates a high activation barrier in solution that prevents equilibration between these structures. Using metadynamics, we explore the conformational energy landscape of Q10 to investigate the physical origins of this high activation barrier. We develop new insights into the conformations and hydrogen bonding environments of the glutamine side chains in the PPII and [beta]-strand-like conformations in solution. We also use the secondary structure-inducing cosolvent, acetonitrile, to investigate the conformations present in low dielectric constant solutions with decreased solvent-peptide hydrogen bonding. As the mole fraction of acetonitrile increases, Q10 converts from PPII-like structures into [alpha]-helix-like structures and [beta]-sheet aggregates. Electron microscopy indicates that the aggregates prepared from these acetonitrile-rich solutions show morphologies similar to our previously observed polyQ fibrils. These aggregates re-dissolve upon the addition of water! These are the first examples of reversible fibril formation. Our monomeric Q10 peptides clearly sample broad regions of their available conformational energy landscape. The work here develops molecular-level insight into monomeric Q10 conformations and investigates the activation barriers between different monomer states and their evolution into fibrils.

**Keywords:** Protein, Spectroscopy

**Application Code:** Bioanalytical

**Methodology Code:** Molecular Spectroscopy
Fibrillation is a naturally occurring phenomenon. During fibrillation the native secondary structure of a protein is reformed towards a \( [\beta] \)-sheet dominated structure. Several protein amyloids deposit plaques in and on cells and tissues leading to severe damage and diseases. Standard fluorescence and spectroscopic techniques to identify amyloids provide only a limited spatial resolution and individual amyloid structures cannot be analyzed structurally. On the other hand, scanning probe microscopy provides information on the morphology but a chemical characterization remains out of reach. Tip-enhanced Raman scattering (TERS)\[1\] allows to characterize e.g. amyloid fibrils of insulin \[2,3\] and even glycoproteins\[4\] on the nanometer scale. TERS provides chemical information of minute volumes directly beneath the tip, which can be correlated with the topography. The focus here will be the nanoscale localization of specific domains and on the inhibition of the insulin fibrillation process.\[5\] Aggregates obtained from insulin fibrillation in the presence of \( [\beta] \)-carotene (or quercetin) will be presented. Under the influence of these inhibitors insulin does not form normal fibrillar structures. Interestingly all TERS spectra of the \( [\beta] \)-carotene treated samples indicate a major contribution of \( [\alpha] \)-helix unordered structures. This is further supported by Raman microscopy experiments that relate to the core composition of the fibrils. Evidently, the natural products at least prevented the formation of well-ordered amyloid fibrils under the selected conditions.

\[1\] e.g. T. Deckert-Gaudig, A. Taguchi, S. Kawata, V. Deckert, Chem. Soc. Rev. 2017, 46, 4077.

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

Application Code: Bioanalytical
Methodology Code: Vibrational Spectroscopy
Vibrational circular dichroism (VCD) has emerged in recent years as a sensitive technique for 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. The principal advantage of VCD is its combination of the structural richness of vibrational transitions with the chiroptical sensitivity optical activity. Over a decade ago it was discovered that VCD exhibits unusual sensitivity to growth and development of amyloid fibrils owing to their previously unknown long-range supramolecular chiral structure [3]. Since then a series of studies has shown that this supramolecular chirality, or helical twist of the fibril filaments, can be reversed and controlled by careful control of pH, and that the sense of helical filament chirality can be correlated with AFM/SEM-characterized fibril morphology, left twisted or flat [4]. More fundamentally VCD has been shown across five different protein/peptide fibrils to be sensitive the fibril chirality at the individual filament level that is below the level of sensitivity of morphological imaging by AFM or SEM. In this presentation we will review progress to date in understanding the origin of specific enhanced VCD features and the relationship of these features to fibril supramolecular chirality.


Keywords: Bioanalytical, Chiral, Infrared and Raman, Peptides
Application Code: Bioanalytical
Methodology Code: Molecular Spectroscopy
Amyloid is an elaborate, elegant, and complex hierarchical material that can function as a protein genetic element. Examination of a pH-sensitive peptide assembly has now revealed a complex series of phase transitions critical for nucleation, elongation and selection of the assembly structure. With transmission electron microscopy, circular dichroism, and isotope-edited Fourier transform infrared spectrometry, experimental analyses reveal metastable intermediates that make possible pH-independent nucleation. These initial nucleated assemblies do not remain stable in solution, but mutate pH-dependently during propagation into different structures. This multi-phase pathway is also observed in dynamic chemical networks (DCNs). Simple di- and tri-peptides containing a C-terminal aldehyde creates the reversible covalent bonding of the network within a peptide backbone that undergoes these amyloid assembly transitions. The initial polydisperse DCNs yield pure monodisperse homochiral polymers through template-directed selection. The common solution-particle-assembly transitions from both systems are simulated with a two-step nucleation kinetic models to generalize the approach for creating these new informational materials.
For many years, the chemistry of sulfur-containing amino acids cysteine and methionine has gained significant attention due to their biological implications in protein structure, aging, and pathogenesis. Disulfide bonds play an important role in the stabilization of protein structure, and their reduction can accelerate protein fibrillation. Amyloid fibrils are associated with many degenerative diseases, including Alzheimer’s, Parkinson, among others. We have reported that hydrogen sulfide (H$_2$S), a neuromodulator whose amount is significantly decreased in the brain tissue of Alzheimer’s disease patients, inhibits fibrillation through a mechanism which involves trisulfide bonds formation. A Raman band at 490 cm$^{-1}$ was assigned to the trisulfide moiety, the formation of which was confirmed with LC-MS. On the other hand, the methionine residue is known for its radical scavenging property. A current hot topic is the one electron oxidation of methionine, which could play a significant role in protein charge transfer. We report here the stabilization of a methionine sulfur radical cation with $\cdot$-electrons from a phenylalanine ring in a unique fibril scaffold. Such stabilization involves a reversible formation of three electron bond and a bright purple chromophore. A combination of complementary techniques including normal and resonance Raman spectroscopy and electron paramagnetic resonance (EPR), was utilized to probe the structure of the chromophore.
New Technologies for Rapid Monitoring of Neurochemicals In Vivo

Studying Single Cell Acetylcholine Release with Scanning Electrochemical Microcopy and NanoITIES Pipet Electrode

Extrasynaptic transmission is critical in organisms’ biological functions. Single cell release of catecholamine have been extensively studied, however little work have been done on acetylcholine (ACh) release. Here, we demonstrate the release of ACh at the single cell level with high spatiotemporal resolution. ACh is not redox active and cannot be detected using a conventional carbon electrode directly. We used a novel liquid-junction nanopipet (nanoITIES pipet) electrode (1, 2, 3, 4) to measure ACh release. The detection is based on ion transfer across polarized liquid junction, where the half-wave detection potential follows Nernstian equation (5) and the current is proportional to the concentration of the analyte.(6) The nanoITIES electrode was positioned using Scanning Electrochemical Microscopy7 over the neuron cell with nanometer spatial resolution. Quantitative information such as extra-synaptic concentration, number of molecules et al. were measured. Scanning electrochemical microscope combined with nanoITIES pipet electrodes are a valuable toolsets for examining the real-time somatic release events at nanometer distance from the live neurons non-invasively, where ACh release can be studied before its dilution that occurs when it diffuses away to the extracellular medium from the release sites on the soma.

Acknowledgement: We are grateful for the financial support from the National Institutes of Health under Award Number R21NS085665.

References:
1. Analytical Methods, 2015, 7, 7095-7105.
5. Electrochimica Acta, 2000, 45, 2647-2662,

Keywords: Bioanalytical, Electrochemistry, Imaging, Neurochemistry

Application Code: Neurochemistry

Methodology Code: Electrochemistry
Researchers now more than ever believe that to understand and diagnose neurological disorders, it is important not only to detect levels of a single chemical in the brain but to simultaneously examine the changing ratios of chemical and electrical signals in the brain. This requires the development of specialized microelectrode arrays (MEAs) and companion electronics that can simultaneously record multiple chemical and electrical signals from tissue over extended periods of time. In pursuit of a device that can achieve this goal, we have developed a flexible MEA that allows for monitoring of long-term local tissue responses in an awake, freely moving animal. Individual electrodes on these MEAs are optimized with respect to the desired functionality and surface modification of an electrode can be used to tailor such properties as interface impedance, interface adhesion, hydrophilicity, biocompatibility and sensor selectivity. While the device itself can now survive for many months in the body, there are several reasons the chemical sensors integrated into these arrays are not yet robust enough to survive more than a few weeks. Here we demonstrate improved lifetimes for different classes of chemical sensors thanks to surface modifications that improve stability of sensor membranes and modified immobilization of sensor components to prevent their breakdown or dissolution. Thanks to these modifications the chemical sensors on our arrays are approaching a functional lifetime that is compatible with the longer lifetime of the arrays themselves. In addition to demonstrating the extended the lifetime of these multi-functional MEAs, we will present examples of the unique discoveries that can come from these types of arrays.

Keywords: Bioanalytical, Biosensors, Electrochemistry, Neurochemistry
Application Code: Neurochemistry
Methodology Code: Electrochemistry
Monitoring the concentration dynamics of neurochemicals in vivo is a vital tool for studying brain function, diseases, and treatments. A versatile approach for in vivo monitoring of brain chemistry is to couple sampling methods, such as microdialysis, to analytical measurements. Although this approach is valuable, its utility is limited by low spatial and temporal resolution. Spatial resolution is important because many brain regions are small. Temporal resolution is important because concentrations of neurotransmitters can change rapidly during behavior. We report microfluidic/mass spectrometry that overcomes these limitations of microdialysis. Current microdialysis probes are assembled from fused silica capillaries and dialysis membranes resulting in probes with 250 µm diameter and 2-4 mm sampling length. We have recently developed techniques to microfabricate probes from Si that are 30 µm x 55 µm to achieve a 30-fold reduction in size and 10-fold improvement in sampling spatial resolution based on membrane area. A 100-fold improvement in spatial resolution has been achieved by microfabricating push-pull probes where sampling only occurs at the probe tip. To achieve high temporal resolution, probes are be equipped with microfluidics to segment samples into droplets to prevent broadening of sampled concentration pulses as they are transferred to an analytical system. Better than 1 s temporal resolution is possible. To analyze the nanoliter samples that are collected, we use nanospray ionization mass spectrometry. Pumping nanoliter droplet samples into a nanospray source at flow rates < 100 nL/min allows detection of several low molecular weight neurotransmitters. These techniques provide unprecedented temporal and spatial resolution for in vivo monitoring of a wide variety of neurochemicals.

Keywords: Lab-on-a-Chip/Microfluidics, Mass Spectrometry, Neurochemistry
Application Code: Neurochemistry
Methodology Code: Microfluidics/Lab-on-a-Chip
New Technologies for Rapid Monitoring of Neurochemicals In Vivo

Wearable Droplet Microfluidic Based Chemical Sensors for Rapid and Continuous Monitoring

Here I report our recent development on droplet-microfluidic based continuous chemical sensors for healthcare monitoring. We demonstrate that droplet-microfluidics is well suited to form a new generation of wearable chemical sensors with advantages on high frequency sampling and real-time analysis.

Continuous measurement of biomolecule/drug concentrations directly from tissue, blood or other body fluids offers the exciting possibility of understanding physiological or pathological processes, recording responses to stimuli, drug metabolism, and even developing new therapies that use biomarker levels to guide treatment in real-time. However, such measurement is challenging - the fluids are complex mixtures, the volumes can be very small, and real-time detection methods are limited.

Here we develop sensor device containing novel micro peristaltic pumps for accurate and robust collection of fluidic sample into compartmentalized droplets with added regents, therefore crucially Taylor dispersion and chemical contamination are avoided, and near real-time assays can be performed. Droplet generation in the device is on-demand. Droplet size is constant, determined by design (the channel and pump dimensions), and is irrelevant to liquid property normally encountered, also not affected by variations from flow rate, different sampling frequency, or whether it is continuous or intermittent sampling.

The device is also equipped with other components including an optical sensor array for colorimetric detection, electronic circuit (for data acquisition and wireless transmission with Bluetooth), SD card and batteries, therefore making it a fully functional and autonomous system.

As example applications, we have successfully tested the sensor in vivo for continuous glucose/lactate monitoring on human subjects.

Keywords: Biosensors, Lab-on-a-Chip/Microfluidics, Monitoring, Sampling
Application Code: High-Throughput Chemical Analysis
Methodology Code: Microfluidics/Lab-on-a-Chip
Measurements of neurochemicals in the extracellular space are limited by combinations of poor chemical, spatial, and temporal resolution. Brain chemistries, therefore, are unable to be investigated dynamically, particularly at the level of neural circuits and across numerous signaling molecules. To understand neural signaling at scales pertinent to encoded information, micro- to nanoscale sensors are needed for multiplexed, highly selective readouts of extracellular neurotransmitter concentrations with sub-second response times. We have designed, developed, and tested sensors that are approaching these critical attributes. Neurotransmitter recognition is by oligonucleotide receptors (aptamers) linked to field-effect transistor (FET) arrays for electronic transduction of reversible binding events via conductance changes. For example, using aptamer-FETs, we have selectively detected serotonin and dopamine over five orders of magnitude with fM detection limits in artificial cerebrospinal fluid. Serotonin was measured in brain tissue at physiological concentrations. Currently, we are investigating and tuning temporal resolution of aptamer-functionalized FETs using microfluidics. We are investigating the impact of different surfaces chemistries on sensitivity and biofouling. We are also lithographically fabricating FETs on silicon microprobes for in vivo testing. Beyond serotonin and dopamine, we are developing sensors for a broad array of monoamine, amino acid, and peptide neurotransmitters.


Keywords: Biosensors, Nanotechnology, Neurochemistry, Semiconductor
Application Code: Neurochemistry
Methodology Code: Sensors
Recent Advances in Two-Dimensional Liquid Chromatography Separations

Development of Active Solvent Modulation as a Versatile Approach to Improve the Compatibility of Different Separation Modes Used in 2D-LC

The inability to couple certain types of separation modes effectively in a two-dimensional (2D) separation format has historically been a significant impediment to wider use of 2D liquid chromatography (2D-LC). In most cases this is a consequence of the properties of solvents used in the two separations being favorable for one separation, but not the other. A well-recognized example involves reversed-phase separations in both dimensions, where the effluent from the first dimension ([sup]1[/sup]D) column containing a high percentage of organic solvent can negatively impact the performance of the second dimension ([sup]2[/sup]D) column.

Recently, we have been working to develop Active Solvent Modulation (ASM) as a valve-based approach to overcome this incompatibility problem. Briefly, the design of the valve used for ASM enables passive splitting of the eluent from the [sup]2[/sup]D pump so that part of it displaces fractions collected by the valve from the [sup]1[/sup]D separation to inject them into the [sup]2[/sup]D column, while the other part of the flow bypasses the valve before rejoining the other stream to effectively dilute the sample prior to injection into the [sup]2[/sup]D column. In this presentation we will describe recent insights on the utility of this approach obtained from both simulations and experiments. Results of simulations help us understand the scope of the ASM approach (i.e., which molecule and separation types is it good for), and the impact on resolution, detection sensitivity, and method development decisions relevant to the [sup]2[/sup]D separation in particular. We will present results of representative 2D-LC separations of peptides and proteins that illustrate the utility of the ASM approach.

Keywords: Food Science, Liquid Chromatography, Liquid Chromatography/Mass Spectroscopy, Pharmaceutical
Application Code: Pharmaceutical
Methodology Code: Liquid Chromatography
Recent Advances in Two-Dimensional Liquid Chromatography Separations

Two-Dimensional Liquid Chromatography Coupled to Mass Spectrometry for Impurity Analysis of Oligonucleotides

The promise of oligonucleotides as therapeutics has long been realized, but advances in modifications to DNA and RNA for enhancing stability and increasing efficacy have helped contribute to a recent resurgence. Although solid-phase oligonucleotide synthesis results in relatively high coupling efficiencies, the overall purity decreases as the oligonucleotide length increases. Ion-exchange chromatography (IEX) and ion-pair reversed-phase (IP-RP) chromatography both serve as common separation modes for assessing impurities. In IEX, separation is largely based on charge, making IEX a suitable method for separating insertions and deletions, but less effective at detecting sequence alterations. As with many IEX methods for biomolecule analysis, the mobile phase used is not readily compatible with mass spectrometry (MS), making impurity identification difficult. In IP-RP chromatography, retention is largely based on length and hydrophobicity of the oligonucleotide, which results in sequence alterations being more readily determined, but similar challenges are faced when selecting a mobile phase to optimize both chromatography and MS results. Amine IP reagents when titrated to their ammonium acetate form offer efficient separations that are in non-denaturing conditions, but MS response is compromised due to ion suppression. Conversely, amines buffered with hexafluoroisopropanol (HFIP) are often incorporated for optimal MS performance, but as a denaturing method, is not always suitable for analysis of modified oligonucleotides.

In this study, modified oligonucleotides will be analyzed using two-dimensional liquid chromatography coupled to mass spectrometry (2D LC-MS) to address the challenges faced when selecting a mobile phase to optimize both chromatography and mass spectrometry. By incorporating a second dimension separation and at-column-dilution, sensitivity is improved to detect impurities that are not readily detectable through one-dimensional chromatography alone.

Keywords: Biopharmaceutical, HPLC, Liquid Chromatography/Mass Spectroscopy, Pharmaceutical
Application Code: Pharmaceutical
Methodology Code: Liquid Chromatography/Mass Spectrometry
Chiral separation by liquid chromatography (LC) has proven to be an enabling technology in drug discovery, clinical diagnosis, food and agrochemistry, environmental analysis and many other fields. A wide variety of chiral columns are nowadays commercially available to cope with the problem of separating stereoisomers in liquid chromatography. However, samples are often complex and there is interest in separating constituents of samples in a comprehensive manner. Single dimension separations are often not sufficient to separate such mixtures or require excessive time (both for method development and analytical runs). New equipment and newest column technologies, however, have become available which allow straightforward and convenient development of multidimensional separations (MDLC) which can overcome both of those problems.

In this presentation the different modalities of enantioselective MDLC, such as offline LCxLC, online multiple heart cutting, high resolution sampling (selective comprehensive), and fully comprehensive LCxLC, will be discussed. Advantages, challenges, problems and how they can be overcome as well as disadvantages of each modality will be outlined by real applications from pharmaceutical analysis to biotechnology.

**Keywords:** Liquid Chromatography, Method Development

**Application Code:** Pharmaceutical

**Methodology Code:** Liquid Chromatography
Recent Advances in Two-Dimensional Liquid Chromatography Separations

Improvements in 2D-LC Applications Enabled by New Instrument Technology

A two-dimensional liquid chromatography (2D-LC) system collects aliquots of effluent from a first dimension (1D) column, which are transferred to a 2D column to separate those compounds that co-eluted in 1D - an idea [1] that took some time to ripen. It was mainly the last decade during which tremendous progress has been made, accelerated by the evolution of both, hardware and software that is specifically designed for 2D-LC. Today, instrumentation is available from several manufacturers.

Dominant operational modes are comprehensive and heart-cutting 2D-LC applied respectively, for untargeted analyses of highly complex mixtures or targeted separations, often of structurally similar molecules.

This presentation will focus on a technique referred to as multiple heart-cutting (MHC) 2D-LC [2,3], which breaks the link between the 1D and 2D separation timescales allowing for operation of both dimensions nearly independently. We will demonstrate the potential of 2D-LC to tackle tough separations and describe recent advances in technology that help to overcome major obstacles in method development. For illustration purposes, application examples from the chemical as well as the pharmaceutical industry are taken to show:

- How MHC 2D-LC enhances the heart-cutting experiment.
- How two 1D-separations with poor resolution can be combined to fully resolve a mixture.
- How HPLC with non-volatile additives is made amenable to mass spectrometry.
- How to improve 2D performance by improving the compatibility of solvent systems in 1D and 2D.


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

Application Code: General Interest

Methodology Code: Liquid Chromatography/Mass Spectrometry
Polymer conjugated small and large molecules are new drug modalities to increase efficacy, improve PK profile and extend the long lasting release, such as PEGylated drugs, PLGA and hydrogel long acting delivery products. The characterization of the polymers is critical for the conjugation process and final drug product quality. These polymers are highly heterogeneous and some of them only have very subtle differences. For instance, maleimide polyethylene glycol is commonly used for protein conjugation. The molecular weight, number of dendrimeric arms, the number of maleimide groups and its associated maleimide hydrolysis impurities all play a critical role in the downstream process and final protein conjugation product. Conventional HPLC method is not able to separate all of these species with different properties. These biocompatible high molecular weight polymers have a highly convoluted charge distribution profile by MS electrospray and prevent the MS identification. To overcome these challenges, we used 2DLC-QTOF with charge reduction mass spectrometry to deconvolute the polymer distributions to lower charge states in order to identify and characterize the heterogenous polymer profile and its maleimide related species. To achieve adequate resolution of all impurities in the complex polymer sample mixture, chemical degradants and process related impurities were separated from the polymer molecules, and the size variant polymer species were separated in the 2nd dimension. Once resolution of each isolated polymer species was achieved by 2D-LC, charge reduction mass spectrometry was then used to identify the resolved peaks through analysis of the reduced charge states and deconvoluted mass spectra.

**Keywords:** Biopharmaceutical, Drug Discovery, Liquid Chromatography/Mass Spectroscopy, Pharmaceutical

**Application Code:** Pharmaceutical

**Methodology Code:** Liquid Chromatography/Mass Spectrometry
Clinicians rely on laboratory data and imaging to guide and complement their diagnosis and treatment of patients. But, there are limitations to monitoring organ function. Blood and urine are preferred as are not invasive. But changes in metabolic profiles and measure drugs or biomarkers are better monitored due to a higher sampling yield of a biopsy, with downside of being more invasive, as well as being associated with more morbidity and complications such as bleeding, and infection. In this presentation, the clinicians' goals for ultimate complementary tests are discussed, specifically focusing on techniques involving the tracking of biochemical alterations on a global view.

**Keywords:** Biological Samples, Liquid Chromatography/Mass Spectroscopy

**Application Code:** Biomedical

**Methodology Code:** Liquid Chromatography/Mass Spectrometry
We have developed an integrated system for the measurement of dopamine and serotonin. Our goal is to make measurements of neurotransmitter dynamics and basal levels continuously or during certain periods over many days in awake, behaving animals. The presentation will describe the theory-guided experimental work that has resulted in new capabilities for microdialysis monitoring.

**Keywords:** Biomedical, Liquid Chromatography, Neurochemistry

**Application Code:** Neurochemistry

**Methodology Code:** Liquid Chromatography
Neuropeptides represent an important class of signaling molecules whose identities and functions are not yet fully understood, in part due to difficulties in studying them using traditional biochemical methods. Mass spectrometry (MS)-based tools enable precisely determining the identity of a neuropeptide or quantifying a compound with a known sequence, while revealing its putative function with in vivo sampling and quantitative analysis. Tools based on MS and tandem MS (MS/MS) have been developed, both with isotope labeling strategies and label-free methods, that allow accurate quantitation of neuropeptide changes associated with behavior or physiological manipulation, concurrent with identification of sequence. MS and MS/MS have also been implemented with sampling methods that incorporate temporal or spatial information while determining putative functional role of a neuropeptide, such as in vivo microdialysis or tissue imaging mass spectrometry. We employ the crustacean stomatogastric nervous system and its associated neuroendocrine organs as a test-bed for technology development and validation due to the unique advantages and biological significance of this model system. In parallel, we aim to translate our technology development for neuropeptide discovery and analysis to the mammalian central nervous system. Equipped with high-resolution accurate mass (HRAM) Orbitrap instrumentation coupled with various separation techniques and isotopic and isobaric labeling strategies, we explore peptidomic changes in the regulation of feeding behavior. Via a combination of cryostat dissection, heat stabilization, neuropeptide extraction and quantitative neuropeptidomics, a diverse group of neuropeptides were found to alter expression levels in response to feeding, suggesting their potential roles in regulation of food intake.
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| Primary Author | Zoltan Takats  
Imperial College London                                                                                                                                                                          |
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**Abstract Text**

Rapid Evaporative Ionization Mass Spectrometry has been demonstrated to be a feasible approach for intrasurgical real-time tissue identification in cancer surgery. The approach combines standard electrosurgical tools with on-line mass spectrometric analysis of the surgical aerosol to provide the surgical personnel with continuous information on the tissues being dissected. The methodology – commonly known as iKnife – utilizes a large histologically annotated spectral database and multivariate statistical analysis for the identification of MS data. However, these database entries have been created by sampling ex-vivo tissue specimens by electrosurgery and subsequent histological assessment of the void space left behind electrosurgical evaporation. This approach has questionable reliability and produces very limited number of data points. Carbon dioxide laser desorption ionization mass spectrometry has been recognized to produce data comparable to the iKnife data, but it’s considerably less destructive. Mass spectrometric imaging using laser desorption ionization was tested as an alternative way to create histologically assigned training data sets for the iKnife. The results obtained for breast cancer clearly show the feasibility of this approach, resulting in improved information recovery and accuracy.

**Keywords:** Imaging, Instrumentation, Mass Spectrometry, Medical

**Application Code:** Clinical/Toxicology

**Methodology Code:** Mass Spectrometry
Tissue analysis is very challenging because of the diversity and heterogeneity of the matrix. There are many established analytical methods for tissue collection and processing, however most of them is labor and time consuming, particularly when multi-target monitoring is of interest. For research purposes, where analysis time per sample as well as invasiveness related to collection of the sample is not the main concern, the protocols based on biopsy or post-mortem studies are routinely used. However, for real-life clinical applications, both overall time “from sample-to-result” and minimum invasiveness are critical.

The overview of the technology, which addresses the aforementioned demands will be presented. Solid phase microextraction (SPME) has been tested for targeted and untargeted tissue analysis for the past few years in animal and human models. In vivo SPME combines sampling, sample preparation, extraction and metabolism quenching into single step, eliminates the use of solvents for extraction thus making it simple and on-site friendly. Moreover, very important feature of the method is extraction of metabolites directly from the tissue with sterile micro-probe without the need of sample removal (so called “chemical biopsy”). This minimizes invasiveness of the procedure and, consequently, possible complications but in the same it provides us with information about tissue biochemistry.

The potential of the technology has been already tested in quantitative drug distribution study in lungs, brain tumor studies, in vivo monitoring of brain chemisry, and metabolomics profiling of kidney, liver and lung grafts to monitor the influence of ischemia, oxidative stress and organs response to different preservation protocols.

In the talk, the characteristics of the method will be discussed along with its future perspectives in biomedical research and clinical practice, particularly as point-of-care diagnostic tool (ICU, OR, ER).
In recent years, single-molecule fluorescence techniques including single-molecule tracking (SMT) have been realized as useful tools to measure the morphologies and physicochemical properties of nanostructured materials. In this presentation, I will introduce our recent SMT studies aiming to understand the properties of individual nanoscale block copolymer microdomains. We record the diffusive motions of individual sulforhodamine B (SRB) molecules incorporated into elongated microdomains in cylinder-forming polystyrene-block-poly(ethylene oxide) (PS-b-PEO) films. Trajectories obtained from one-dimensionally diffusing molecules provide a means to measure the diffusion coefficients and diffusion directions of single SRB molecules, which correspond to the permeability and orientation of individual SRB-incorporating PEO microdomains, respectively. SMT and fluorescence recovery after photobleaching (FRAP) measurements at identical µm-scale areas permit us to verify the ergodicity in single-molecule and ensemble-averaged diffusion behavior with minimum influence of material heterogeneity. Furthermore, the influence of solvent-induced swelling on permeability and radius for individual microdomains can be quantitatively assessed using SMT. These fluorescence techniques give valuable information on the morphology and permeability of individual polymer nanostructures under ambient conditions that will be relevant to the engineering of polymer nanostructures suitable for chemical separations and sensing. This work is supported by the US-DOE (DE-SC0002362).
State-of-the-Art Optical Microscopy for Polymer Nanostructure Characterization

Scanning Electrochemical Microscopy Meets Raman Spectroscopy: Elucidating Charge Storage on Nanoscale Assemblies Using a Versatile Spectroelectrochemical Probe

New nanoelectrochemical, in situ, surface sensitive, and spatially-resolved tools are required for elucidating the various concurrent processes at electrodes used for energy storage. We will describe efforts in our group to integrate these capabilities on a versatile imaging, measurement, and spectroelectrochemical platform based on scanning electrochemical microscopy (SECM).

We will discuss the coupling of Raman spectroscopy to a SECM with the intention of obtaining simultaneous electrochemical and spectroscopic information. In a first application, the redox properties of viologen-based redox active colloids (Vio-RACs)[1,2] were investigated using bulk and single particle electrochemical methods. RACs are cross-linked polymers with well-defined size and shape. Nanoelectrochemical examination allowed to quantitatively determine the intra-particle concentration of viologen. Further examination using Raman showed the absence of charge trapping, and demonstrated the use of viologen as a simultaneous Raman and redox probe. The combination of Raman and SECM was performed through the use of the Surface Interrogation mode (SI-SECM), where we followed quantitatively the titration of viologen pendants on monolayer assemblies of RACs. In a second application, we will discuss our efforts in exploring the modulation of graphene reactivity by local electronic structure perturbations. Experiments designed to tackle single-particle and single-site reactivity are shedding new light on the rate-limiting steps during energy storage. The unique combination of SECM schemes and the ability to obtain simultaneously electronic structure information adds exciting capabilities to an emerging toolbox for correlating battery electrode structure and reactivity.


Scanning angle (SA) Raman spectroscopy uses a prism optically coupled to a thin polymer film to collect Raman spectra as the incident angle of an excitation laser is varied with 0.05 degree precision. A SA Raman spectroscopy method was developed to measure the location of buried interfaces between polymer layers in multilayer waveguide films and to simultaneously measure refractive index and film thickness in thin mixed-polymer films. Six multilayer films consisting of poly(methyl methacrylate) (PMMA) and polystyrene (PS) were prepared with total thicknesses ranging from 330-1260 nm, and the interface locations were varied by altering the individual layer thicknesses. The Raman amplitude ratio of the PS and PMMA peaks was used in calculations of the electric field intensity within the polymer layers to model the SA Raman data and extract the total thickness and interface locations. In addition, polymer films formed by spin coating the block co-polymer PS-b-PMMA and mixtures of the block copolymer and PMMA were formed. The amount of PMMA in the solution was varied to form polystyrene domains of varying size and the spin coating conditions were varied to alter the polymer thickness. The data show that SA Raman spectroscopy can be used to accurately determine thickness (as confirmed with optical profilometry) and chemical composition (as confirmed with fluorescence microscopy). There is an increasing demand for nondestructive in situ techniques that measure chemical content, total thickness, and interface locations for multilayer polymer films, and SA Raman spectroscopy in combination with appropriate data models can provide this information.

This research is supported by the U.S. Department of Energy, Office of Basic Energy Sciences, Division of Chemical Sciences, Geosciences, and Biosciences through the Ames Laboratory. The Ames Laboratory is operated for the U.S. Department of Energy by Iowa State University under Contract No. DE-AC02-07CH11358.

Keywords: Microscopy, Vibrational Spectroscopy
Application Code: Polymers and Plastics
Methodology Code: Microscopy
To thoroughly elucidate how molecular conformation and photophysical properties of conjugated polymers are related requires simultaneous probing of both. First, we employ algorithms based on the super-resolution technique known as single molecule high resolution imaging with photobleaching (SHRImP) to localize individual emitters along single conjugated polymers. SHRImP processing corroborates that compact poly(2-methoxy-5-(2-ethylhexyloxy)-1,4-phenylenevinylene) (MEH-PPV) molecules have distinct photophysical properties from extended ones and yields estimated radii of gyration in agreement with theoretical predictions. Towards understanding such molecules in the context of their environment as it exists in devices, we then utilize a multi-modal apparatus that allows for controlled solvent vapor swelling and simultaneous wide-field epi-fluorescence microscopy to demonstrate bottom-up growth of morphologically ordered anisotropic aggregates prepared from single MEH-PPV chains. We quantify aggregate physical and optical anisotropy, degree of quenching, and exciton diffusion characteristics as a function of aggregate size. Moreover, real time monitoring of the aggregates during growth demonstrates that aggregate assembly occurs through multiple mechanisms including both Ostwald ripening and aggregate coalescence. These growth mechanisms have distinct and strong impact on the evolution of physical and optical anisotropy of the aggregates: while Ostwald ripening templated on highly ordered single molecules allows preservation of the highly ordered morphology, aggregate coalescence occurs with no preferential orientation, leading to an attenuation in physical and optical anisotropy. We suggest that propensity for a particular growth mechanism can be tuned, providing a path for controlled processing of conjugated polymer aggregates at length scales relevant to the operation of devices.
Confined polymeric environments, such as interfaces, brushes, and gels, may exhibit spatial heterogeneity for many reasons, including local chemical or structural inhomogeneity, or topographical effects. We have developed an approach to obtain spatial maps of these heterogeneities based on the premise that “probe” molecules exhibit varied behavior that is distinctively related to the local chemistry and topology. This includes dynamic behavior (e.g. adsorption rate, residence time, and local molecular mobility), molecular conformation, as well as photophysical phenomena (e.g. fluorescence emission). All of these are exquisitely sensitive to the details of the local environment.

By choosing appropriate fluorescently labeled probe molecules and accumulating large numbers of dynamic super-resolution molecular trajectories, the molecular behaviors can be partitioned and used to prepare spatial maps, which can be calibrated by comparison with well-defined control samples. Having identified chemically- or physically-distinct regions, the behavior of molecules of interest can then be partitioned by region. For example, the surface residence time of polymers and proteins can be measured simultaneously as a function of the local hydrophobicity within polymer brushes, or polymer surface diffusion can be compared in the vicinity of different regions of diblock copolymer films. This information can have important technological implications. For example, anomalously strong nanoscale binding sites can be identified on polymeric materials (e.g. biomaterials, filtration membranes, chromatographic media), enabling one to determine their influence on surface fouling and/or separation efficiency.

Keywords: Chromatography, Materials Characterization, Microscopy, Polymers & Plastics
Application Code: Material Science
Methodology Code: Microscopy
The Role of Ultrahigh-Resolution Mass Spectrometry in the “Omics” Era

An Olio of Omics: Nature’s Chemical Compositional Complexity Resolved and Identified by Ultrahigh Resolution Fourier Transform Ion Cyclotron Resonance Mass Spectrometry

The NHMFL 21 T Fourier transform ion cyclotron resonance mass spectrometer [1] offers nonpareil broadband mass resolution and mass accuracy (e.g., more than 125,000 elemental compositions from a single mass spectrum). We shall describe applications ranging from top-down MS/MS [2] for identifying and sequencing proteoforms (proteomics; glycomics) to petroleomics (environmental spills; deposits; emulsions; corrosion; asphaltenes) and other complex mixtures (lipidomics; metabolomics, bio-oils). 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.


The Role of Ultrahigh-Resolution Mass Spectrometry in the “Omics” Era

Top-Down Fourier Transform Mass Spectrometry of Proteins and Protein Complexes as a Tool for Structural Biology and Drug Development

Native mass spectrometry (MS) of proteins and protein assemblies reveals size and stoichiometry. But elucidating their structures to understand their function is more challenging. Membrane proteins are involved in many biological functions, but their characterization by a variety of techniques is challenging. We show that MS can be effective for deriving structural information for soluble and membrane protein complexes. We use top-down high resolution Fourier transform ion cyclotron resonance (FT-ICR) MS to probe ligand-binding sites and to generate topological information for large proteins and complexes. A 15-Tesla FT-ICR instrument with an infinity cell and with nanoelectrospray ionization (nanoESI) was used in this study. Electron capture dissociation (ECD), infrared multiphoton dissociation (IRMPD), and electron ionization dissociation (EID; bias voltage of 30 V) were used with the FT-ICR system.

ESI’s gift for transforming solution-phase macromolecules into gas-phase ionized counterparts without disrupting weak noncovalent interactions is key for applying MS to study protein complexes. Native top-down MS generates information on the surface topology, ligand binding sites, and post-translational modifications of protein complexes. The various activation methods available with FT-ICR MS allow membrane proteins to be measured with high accuracy. We are using native MS/MS to investigate the molecular action of compounds that prevent amyloid fibril formation in neurodegenerative diseases such as Alzheimer’s and Parkinson’s disease.

Abstract Text

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Keywords: Biopharmaceutical, Mass Spectrometry, Protein, Tandem Mass Spec
Application Code: Bioanalytical
Methodology Code: Mass Spectrometry
Novel biomarkers are urgently needed for early detection and prognosis of various diseases, especially when aiming for individualized treatment. Mass spectrometry (MS)-based proteomics and metabolomics have been applied as a discovery tool in retrospective studies of clinical cohorts of body fluids such as serum samples. Clinical MS strategies aiming for diagnostic purposes require robust and high-throughput (HT) platforms. Until now post-translational modifications (PTM’s) have often not been taken into account because of technical limitations and the increased complexity of the resulting data. One of the most common PTM’s is protein glycosylation. The vast majority of membrane and secreted proteins are known or predicted to be N- and/or O-glycosylated. Glycoproteins represent key molecules in many important biological processes, including diseases. In-depth approaches to determine site-specific protein glycosylation have become indispensable tools for functional analyses of these complex biomolecules. Discovery of glycoprotein markers is either pursued on the level of a single protein by mapping all its proteoforms, or by HT glycomics approaches. With regard to the latter the total serum N-glycome (TSNG) comprises the N-glycans from all serum proteins, which are to a large extent liver- (acute-phase proteins) and plasma cell-derived (antibodies). Recent developments in MS-based HT glycosylation analysis have provided the opportunity to acquire information on TSNG N-glycan complexity, antennarity, galactosylation, fucosylation, as well as on the presence and linkage of sialic acids (\(\alpha_{2,6}\) versus \(\alpha_{2,3}\)-linkage). In this presentation we will show examples of clinical glycomics studies that explore the potential of total serum N-glycome analysis as a prognostic panel. Our HT glycomics approaches will be discussed in the context of further developments in the exciting new field of clinical MS (ECgrants HighGlycan, GlyCoCan and GlySign).
The Role of Ultrahigh-Resolution Mass Spectrometry in the “Omics” Era

Top-Down Proteomics Enabled by Ultra-High Resolution Mass Spectrometry and Nanotechnology

Top-down high-resolution mass spectrometry (MS)-based proteomics is arguably the most powerful method to comprehensively characterize proteoforms that arise from genetic variations, alternative splicing, and PTMs. With unmatched mass resolution and accuracy, Fourier transform ion cyclotron resonance (FTICR)-MS has played a significant role in top-down proteomics. We have shown that top-down high-resolution MS has unique advantages for unraveling the molecular complexity, quantifying multiple modified protein forms, complete mapping of modifications with full sequence coverage, and discovering unexpected modifications. However, the top-down approach still faces significant challenges in terms of protein solubility, protein separation, the detection of large and low-abundance proteins, and the under-developed data analysis tools. Herein, we are employing a multi-pronged approach to address these challenges in a comprehensive manner by developing new MS-compatible surfactants for protein solubilization, novel materials and new strategies for multi-dimensional chromatography separation of proteins, novel nanomaterials for enrichment of low-abundance proteins, and a new comprehensive software package for top-down proteomics. Recently, to address the challenges in detection of large proteins, we have developed a novel top-down proteomics platform that couples serial size exclusion chromatography and high-resolution top-down MS for detection and characterization of high molecular weight proteins (>220 kDa) from complex mixtures. In this talk, I will present our recent technology developments in top-down mass spectrometry-based proteomics and its application to cardiac systems biology.

Keywords: Liquid Chromatography, Liquid Chromatography/Mass Spectroscopy, Mass Spectrometry, Membrane
Application Code: Genomics, Proteomics and Other 'Omics
Methodology Code: Mass Spectrometry

Session Title
Abstract Title
Primary Author
Ying Ge
University of Wisconsin-Madison
Co-Authors

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The Role of Ultrahigh-Resolution Mass Spectrometry in the “Oomics” Era

High-field Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) enables ultrahigh mass resolving power and mass measurement accuracy, which is ideal for molecular characterization of complex mixtures such as crude oil, bio-oils, and natural organic matter, and has stimulated rapid development of petroleomics during the last decade. In this work, we report state-of-the-art analysis of complex petroleum mixtures by 21 Tesla FT-ICR MS. Direct infusion mass resolving power is greater than 2 million at m/z 400, and mass measurement accuracy is typically 100 ppb rms, which are more than 2-fold better than prior results at lower magnetic field. Use of offline separation strategies minimizes ionization suppression, which facilitates detection and elemental composition assignment for thousands of additional molecules. The unique combination of resolving power, scan rate, and spectral dynamic range allows online chromatographic coupling to the 21 T FT-ICR system. Mass resolving power greater than 1.5 million (at m/z 400) is achieved with a detection period of 3.1 seconds, with mass measurement accuracy ~300 ppb rms, dynamic range approaching 1000:1 for a single scan, and molecular structure indicated by elution period. The unprecedented analytical performance will elucidate the molecular mechanisms relevant to oil production and refinery deposits, emulsions, corrosion, and upgrading. The 21 T instrument is a part of the National High Field FT-ICR User Facility at the National High Magnetic Field Laboratory, and is available to all qualified users.

Keywords: Ion Cyclotron Resonance, Liquid Chromatography/Mass Spectroscopy, Mass Spectrometry, Petroleum
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Mass Spectrometry
## Abstract Text

Fluctuating ion distributions in biological and environmental systems are difficult to map in real time. Ion optodes are an obvious solution, but many transduction principles rely on the detection of two ions at the same time, making this approach difficult to establish. Electrochemical approaches require either a moving tip, as in scanning electrochemical microscopy, which requires complex instrumentation and loses critical time information, or massive arrays where each electrode needs to be separately interrogated.

This talk will introduce the concept of closed bipolar ion-selective electrodes to solve this long standing problem. Here, each ion-selective electrode triggers an optical signal change at its opposite side that depends on the concentration of the ion to be sensed. This principle can be integrated into a massive array more easily as electrochemical control occurs globally with a single potentiostat, without requiring direct electrical contact to each electrode.

**Keywords:** Electrochemistry, Electrodes, Environmental/Biological Samples, Ion Selective Electrodes

**Application Code:** Environmental

**Methodology Code:** Sensors
Inkjet printing is capable of depositing minute liquid droplets onto various substrates in a flexible, reproducible manner. Even the simplest inkjet printers are equipped with at least 4 ink reservoirs (cyan, yellow, magenta, black) to achieve color printing according to the CYMK color scheme. Ink ejection volumes from each reservoir are controlled by the CMYK color values using computer graphic software. Although this feature has been previously made use of in research applications related to analytical chemistry, the non-linear correlation between the user-defined CMYK print color values and the actually dispensed ink volumes has prevented applications, where the precise control of multiple reagent ratios is crucial.

In this work, a new printing color profile has been elaborated to achieve a linear relationship between set CMYK color values and actually dispensed amounts of inks. One application example demonstrated here is the determination of binding stoichiometries of chromogenic ion indicators according to the Job plot method. In contrast to the conventional method involving manual preparation of multiple solution mixtures, the elaborated inkjet-based approach allows automated mixing of solutions of metal salts and indicators in various ratios on a paper substrate by simple printing. Inkjet-deposited liquid volumes and mixing ratios were controlled by color value settings on a PC. The inkjet-based Job plot method was validated by comparison with binding stoichiometries determined for five metal ion-indicator systems based on the conventional solution approach. Simple scanning and color analysis of filter paper gave accurate results for five different stoichiometries and colorimetric response patterns. This quantitative multi-color inkjet printing approach allows flexible deposition and mixing of solutions, and is thus expected to be useful for a wide range of applications.

Keywords: High Throughput Chemical Analysis, Lab-on-a-Chip/Microfluidics, Paper/Pulp, Sensors
Application Code: Other
Methodology Code: Sensors
## Ionophore-Based Chemical Sensors II

**Ionophore-Based Nanosensors for Gel Based Blood Electrolytes Detection**

Blood electrolytes (typically K+, Na+, Ca2+, Cl- and so on) measurements have become a routine in clinical laboratories to help diagnose a wide variety of acute and chronic illnesses. Ion-selective electrodes (ISEs) for blood electrolytes measurements in modern analyzers require calibrations and careful maintenance to avoid clot. For instance, the ISE module in the latest analyzer Cobas® 8000 from Roche is recommended for use in serum and plasma while blood dilution is still required for indirect ISEs methods. Generally, advanced sensing technology for blood electrolyte detection is in high demand.

Here, we present an optical method to measure blood electrolyte (Na+, K+ and Cl-) based on nanosensors trapped in a hydrogel. The nanosensors are composed of organosilica matrices and are modified with ionophores, ion-exchangers and chromoionophores. The analysis could be performed with digital cameras as well as UV-visible spectrometers or microplate readers. Direct measurement in undiluted human blood was demonstrated.

| Keywords: | Biomedical, Clinical/Toxicology, Environmental/Biological Samples, UV-VIS Absorbance/Luminescence |
| Application Code: | Clinical/Toxicology |
| Methodology Code: | UV/VIS |
We have previously shown that the equilibration time and standard potential of SC ISEs with galvanostatically deposited PEDOT(PSS) as SC depend on the substrate electrode (Au or GC vs Pt) as well as the thickness of the PEDOT(PSS) layer. Equilibration times of SC K+ ISEs were around ~77 min on Pt but less than 10 min when built on Au. It was also found that the standard potentials of the ISEs on Pt were ~150 mV more positive than on Au. SR-XPS studies of PEDOT(PSS) films showed that the PSS-/PEDOT ratio is much larger on Pt (1.16) than on Au (0.24). The higher standard potential and SR-XPS data suggest that the PEDOT(PSS) film on Pt is more hydrophilic which has been confirmed by contact angle measurements. To confirm the validity of our interpretation concerning the source of the differences in the response characteristics of these ISEs on Pt and Au, the mass increase during polymerization of PEDOT(PSS) on Pt and Au electrodes was monitored by a quartz crystal microbalance (QCM). The rate of PEDOT(PSS) polymer growth on Au was much faster in the initial 15 seconds of electrodeposition than on Pt, after which the rates of both approach the same value. The faster rate on Au during this short interval ultimately produced a film on Au that was almost twice as thick as on Pt, which was confirmed by XPS etching studies. More so, the frequency and potential recorded by QCM-potentiometry during equilibration of PEDOT(PSS)-coated substrates show that Pt quickly reaches a stable frequency and potential while the frequency on Au continues to decrease over 24 hours and its potential stabilizes only after 10 hours. PEDOT(PSS) films of the same thickness (different polymerization time) were prepared on Au and Pt to determine if the observed chemical and structural differences were related to the different polymer thicknesses or exclusively the substrate electrode material.

FedEx Institute of Technology and Instrumentation Laboratory generously provided funding for this project.

Keywords: Electrochemistry, Potentiometry, Sensors, Surface Analysis

Application Code: Biomedical

Methodology Code: Sensors
A novel solid contact type for all-solid-state ion-selective electrodes is introduced, yielding high stability and reproducibility of potential readings between sensors as well as improved analytical performance. The transducer phase herein proposed takes advantage of the presence of porphyrinoids containing the same metal ion at different oxidation states. In contrast to the traditional approach, the compounds of choice are not a redox pair, although they have different oxidation states, they cannot be electrochemically driven one to another.
Electrochemical oxidation of polyaniline (PANI) at a neutral pH may be utilized as an electrochemically controllable proton pump in important analytical applications such as automated acid-base titration systems, while electrochemical oxidation of poly(aniline-co-o-aminophenol) [PANOA] may involve electrocatalysis by environmentally important species such as arsenic(III) and arsenic(V) providing a possible pathway for the electroanalysis of these species. In this paper, we have used synchrotron radiation-X-ray photoelectron spectroscopy (SR-XPS), near edge X-ray absorption fine structure (NEXAFS) and valence band spectroscopy (VBS), as done previously with ferrocene doped polymer membranes [1] and electropolymerized poly(3,4-ethylenedioxythiophene):polystyrenesulfonate (PEDOT[PSS]) [2], in the elucidation of the mechanistic pathways for the electrochemical oxidation of PANI and PANOA. For example, with the sub-Coulombic reaction chemistry of PANI at a neutral pH, we have determined the degree of charge transfer of this reaction chemistry, so as to make the chemistry useable at a neutral pH, while a rigorous study of the coupling of the arsenic(III)/arsenic(V) redox half-cell with the electrochemical oxidation of PANOA will provide a much-needed mechanistic insight, so as to enable a usage of this reaction chemistry in the electroanalysis of arsenic in environmental samples.

REFERENCES

Keywords: Electrode Surfaces, Electron Spectroscopy, Ion Selective Electrodes, Surface Analysis
Application Code: Other
Methodology Code: Surface Analysis/Imaging
Conventional ion-sensing membranes (ISMs) are passive (i.e. non-activatable), have low sensitivity (low signal-to-noise ratio), and lack the robustness required to operate in biological systems. Therefore, to overcome these limitations, current state-of-the-art ISMs that are activatable and controllable have been proposed by integrating photoactive compounds (photoacids generators or spiropyrans). However, these compounds either irreversibly dissociate protons[1] or requires an external proton source[2], are activated by ultraviolet light (has low cell penetrating ability and causes cellular damage), and exhibits photodegradation[3]. Our research group focuses on the use of mPAHs coupled to ISMs to surmount these barriers[4,5]. The properties of mPAHs are ideal, since it provides the reference ion (protons) for ion-exchange process after visible-light activation, and the photodissociated state is thermodynamically reversible and long-lived. Towards effectively improving this activatable, controllable and robust ion-sensing platform for biological applications, we now need mPAHs with fluorescence capability, higher pKa’s, and longer activation wavelengths. Herein, we will discuss the fundamental physicochemical properties of mPAHs through theoretical calculations and experimental analysis. Thus, yielding the next generation of novel mPAHs suitable for ISMs for biological and environmental applications. This transformative research represents a true breakthrough in activatable and controllable ion-sensing technology.


Keywords: Biosensors, Sensors
Application Code: Bioanalytical
Methodology Code: Sensors
The development of optical pH sensors for measuring intracellular pH is highly useful to studies of cellular biology as well as applications in diagnostics and therapeutics. However, currently most available pH-sensitive nanoprobes suffer from photobleaching and autofluorescence background in biological samples. We will report of progress towards a pH nanosensor that avoids these problems by taking advantage of near-infrared excitation with the use of upconversion nanoparticles (UCNPs) that emit visible light when excited with a 980 nm laser. Monodisperse particles of pure hexagonal phase with diameter <45 nm are used in conjunction with pH sensitive dyes. This nanosensor is based on energy transfer between UCNPs and pH-dependent anthraquinone dyes. Due to the advantages of upconversion photoluminescence, the nanosensor has the potential to be used for detection of pH in in vitro and in vivo applications. The application is a nucleic acid amplification test for infectious disease is discussed and its use in the cell to follow the pH in the vacuole during endocytosis.

Keywords: Bioanalytical, Fluorescence, Ion Selective Electrodes, Sensors
Application Code: Bioanalytical
Methodology Code: Sensors
Analytical Applications in Material Science

Organophosphate Sensing 2D Photonic Crystal Protein Hydrogels and Organogels

2D Photonic Crystal (2DPC) Protein hydrogels and organogels are novel color changing chemically responsive materials. These pure crosslinked protein materials take advantage of evolutionarily evolved specific protein-ligand binding and enzymatic reactions to generate macroscopic volume phase transitions (VPT). The VPT results from changes in the free energy of the system that induce movement of the mobile phase into or out of the polymer network. The particle spacing of the embedded 2DPC change with the polymer volume. Particle spacing changes shift the wavelength of light diffracted from the 2DPC, which appears as a color change. Protein organogels have the revolutionary advantage that they can utilize low vapor pressure solvents like ethylene glycol. The mobile phase of the low vapor pressure organogels will not evaporate, thus enabling gas sensing over long time periods. The enzymes Acetylcholinesterase and Organophosphorous Hydrolase were used to fabricate stable 2DPC sensing materials that are able to detect and decontaminate organophosphate molecules.

Keywords: Biosensors, Material Science, Pesticides, Sensors
Application Code: Material Science
Methodology Code: Physical Measurements
Powerful analytical tools are required by the microelectronics industry to study buried interfaces in situ, especially when investigating adhesion. Adhesion is incredibly important in the microelectronics industry and very difficult to study. There are many surfaces that must be connected by adhesives, making a suitable multipurpose adhesive difficult to design. Improving the strength and understanding the mechanisms that are involved in adhesion is the focus of many researchers. Methods of increasing adhesion include the use of silane adhesion promoters (SAPs) and plasma treatment of surfaces, but fully understanding these systems is challenging and the right tool is needed. Sum frequency generation (SFG) vibrational spectroscopy is capable of providing molecular structural information of buried solid/solid interfaces in situ nondestructively with sub-monolayer sensitivity. This technique has been utilized to investigate water adsorption at polymer/model-underfill interfaces, with various adhesion promoters in the presence of hygrothermal treatment, to elucidate the molecular mechanisms of adhesion loss. Plasma effects were also studied to determine if covered surfaces behave different at the center and edge positions and how disordering, caused by plasma treatment, can increase adhesion strength. Molecular mechanisms of adhesion enhancement in situ, at buried interfaces were investigated in this research. It was found that silanes migrate to the buried polymer/adhesive interface and can cause changes at the interface resulting in increased adhesion strength. Plasma was found to disorder the polymer/epoxy interface, which then increased the adhesion strength. Systems were also subjected to hygrothermal treatment and it was found that moisture moves to the interface and decreases the adhesion but this can be prevented by SAPs. SFG is a unique and powerful tool that can elucidate molecular structures of buried interfaces related to microelectronics nondestructively.
Poly[(R)-3-hydroxybutyrate-[i]co[/i]-(R)-3-hydroxyhexanoate] (PHBHx) is a bio-based polyester made from bacteria fermentation. It has received attentions due to the biodegradability and desired physical properties. By incorporating HHx unit, the crystallinity of PHBHx can be decreased making it the material with improved physical properties. Systematic studies on single crystals (SCs) of PHBHx have not been given much attention. Because lamellar crystals are the most basic consisting unit for complex crystalline texture, understanding the crystallization behaviors are important to understand crystalline structure.

SCs of PHBHx(3.9mol%) have been grown from solution at (T[sub]c[/sub]) from -20[degree]C to 75[degree]C. XRD and TEM have shown that all the crystals take the alpha crystalline form of homopolymer PHB. Each single crystal appears as needle shape with 4-5nm thick and ED pattern suggest that the growth direction is along the [i]a[/i]-axis. The crystals show a thermal dependent anisotropic growth habits: the growth rate along the [i]a[/i]-axis 70 times faster than that along the [i]b[/i]-axis at low [i]T[/i] , whereas this ratio declines to 20 times at high temperature. Surface energy calculation has been done to explain this anisotropic habit. It turns out that (100) and (010) have energy of (81.75 mJ/m[sup]2[/sup]) and (34.10 mJ/m[sup]2[/sup]), respectively.

SCs of PHBHx have also been grown as a function of HHx content from 3.9 to 13mol%. AFM-IR has been used to obtain the IR spectra of PHBHx single crystals. Preliminary studies indicate that all the single crystals of PHBHx are composed by two phases supported by the amorphous band 1740 cm[sup]-1[/sup] and the crystalline band 1722 cm[sup]-1[/sup]. In addition, peak ratio of 1740 cm[sup]-1[/sup] and 1720 cm[sup]-1[/sup] is a function of HHx content for each individual lamellae.

Keywords: Infrared and Raman, Materials Characterization, Vibrational Spectroscopy
Application Code: Material Science
Methodology Code: Vibrational Spectroscopy
Calcium hydroxide is one of the hydration products of Portland cement. The presence of calcium hydroxide and its high alkalinity in concrete helps in corrosion resistance in the embedded steel structures. Enough amount of calcium hydroxide is important for the passivity of the steel structures in concrete. A new method based on the first overtone of the OH stretching of the OH groups attached to calcium hydroxide that gives rise to an absorption at 7082 cm⁻¹ is used in the quantitative determination of calcium hydroxide in cement. Four concrete mixtures with different water/cement ratio were prepared in the laboratory and allowed to harden over 28 days. Small portions of the concrete samples were crushed, ground into fine powder and their NIR spectra were recorded using a Perkin Elmer an NIR spectrum one equipped with a transflectance accessory. A total of 16 scans at a resolution of 16 cm⁻¹ were made on each sample. The 4th derivative profiles of the spectra were used in the quantitative analysis. An external standard of calcium hydroxide was used as a reference. The results show that the amount of calcium hydroxide formed during hydration of the cement increases with the increase in water/cement ratio and reaches up to 18% in the concrete.
A key limitation of glucose sensors in physiological studies is the potential biofouling or biodegradation when used in complex biological matrices such as serum or urine, and particularly in cells where high levels of protease are present. Fluorescent noble metal nanoclusters have recently emerged as a highly potential label over traditional quantum dots in many research areas. However, the use of nanoclusters in biomedical research is still hindered by the complexity of biological matrices and also the degradation of stabilizing-ligands. In this study, a novel biosensor assembly was developed for the detection of glucose in biological matrices based on novel nanocluster encapsulated porous lipid nanoshell. Under physiological condition, glucose oxidase functionalized gold nanocluster was synthesized and the enzyme-nanocluster complex (GOX-AUNC) was subsequently encapsulated into porous lipid nanoshell. The formulated GOX-AUNC lipid nanoshell remained active and catalyzed reaction of glucose to produce H2O2 which quenched quantitatively the fluorescence of GOX-AUNC. The AUNC fluorescent quenching was proportional to the concentration of glucose in physiological range and provides excellent selectivity for glucose over other sugars and most biological species present in serum. The unique combination of high sensitivity and good selectivity of this biosensor indicates its potential for direct analytical applications and also suggests promising research opportunities using porous lipid nanoshell format.

Keywords: Bioanalytical, Biosensors, Material Science, Membrane
Application Code: Material Science
Methodology Code: Fluorescence/Luminescence
The separation processes take place within the chromatographic column, which contains the stationary phase attached to a support material. In HPLC, silica is the most widely used support material to attach the stationary phase. The use of superficially porous (SP) silica particles provides high chromatographic efficiencies with analysis times comparable to sub-2μm particles, but with lower backpressures. To achieve chromatographic selectivity, the surface of the silica particles is modified to contain a moiety of interest. Herein, we report the modification of SP silica particles containing an amino-phenyl phase. The aminated silica surface can be further functionalized to increase hydrophobicity. The modified SP particles have been characterized by infrared spectroscopy (IR), thermogravimetric analysis (TGA), packed into columns, and tested under HPLC conditions to assess the retention behavior of this stationary phase. Details of the particle modification, as well as the preliminary chromatographic characterization will be presented.

Keywords: Chromatography, HPLC, Materials Characterization, Modified Silica
Application Code: Material Science
Methodology Code: Liquid Chromatography
Analytical Applications in Material Science

Fabrication and Chromatographic Evaluation of Porous Aluminosilicate Polymers and Monoliths

The induction of higher and interconnected porosity in materials can lead to the advancement in their applications in various fields (e.g. catalysis, drug delivery, and separation science). Inorganic polymers have been synthesized using natural aluminosilicate precursor under basic conditions. The porosity has been fabricated using porogens such as cationic surfactants and organic polymer beads. The aluminosilicate polymers have been characterized by SEM, TEM, EDS, FT-IR, XRD and Surface and Porosimetry Analyzer. Surface areas ranging from 60-107 m²/g have been achieved. Moreover, these inorganic polymers have shown to be base stable. The synthesis and evaluation of material as a solid support in liquid chromatography is discussed.

Keywords: FTIR, Liquid Chromatography, Material Science, X-ray Diffraction
Application Code: Material Science
Methodology Code: Liquid Chromatography
As samples continue to increase in complexity, column technology continues to be a key area of research in the field of high performance liquid chromatography (HPLC). Columns with new selectivity and increased stability provide chromatographers with the means to analyze more diverse samples, increased flexibility in method development, and longer lasting columns. For these purposes, the development of new approaches to stationary phases for HPLC is an active area of research. We have synthesized a polymeric phenylene layer containing amino groups on superficially porous silica particles yielding candidate materials with the potential to be effective HPLC stationary phases. The synthesized bonded phases were characterized through various techniques, including elemental analysis, diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS), and x-ray photoelectron spectroscopy (XPS). Spectroscopic data combined with electron microscopy allowed for an in depth analysis of the surface generated. Additionally, an assessment of the chromatographic properties of the generated material was performed. This includes determination of the silanol activity, hydrolytic stability, and the molecular interactions responsible for retention via the linear solvation energy relationships model. The amine groups contained in the polymer can potentially act as adsorptive sites in HPLC and also provide a means to further functionalization if desired. Herein, the results of both the spectroscopic and chromatographic testing of the columns generated will be presented.

Keywords: Chromatography, HPLC, Liquid Chromatography, Material Science

Application Code: Material Science

Methodology Code: Liquid Chromatography
Remote sensing techniques coupled with an automated classifier enable the rapid detection of chemical vapors during emergency response scenarios such as chemical plant accidents or natural disasters. The objective of this project is to develop a classifier for the automated detection of methanol vapor from passive infrared multispectral imaging data. Data used in this work were collected with an infrared line scanner mounted in a downward-looking mode on a fixed-wing aircraft operated by the U.S. Environmental Protection Agency Airborne Spectral Photometric Environmental Collection Technology program. The instrument was equipped with eight infrared bandpass filters in the spectral range of 8-14 μm. A rotating prism allowed a 60º arc to be sampled across the flight track of the aircraft. Internal blackbodies mounted at the extremes of the scan track allowed the detector signals to be calibrated to spectral radiance units. The classification strategy was based on the use of a backpropagation neural network in which the network inputs were ratios of the calibrated band intensities. Topics to be discussed during the presentation include (1) strategies for assembling the training data required for computing the network, (2) data preprocessing methods for removing the effects of variation in ground temperatures, and (3) feature selection strategies for identifying the most useful band ratios. The classification methodology will be demonstrated using data acquired from aircraft overflights made during controlled releases of methanol plumes from a portable emission stack. The ability of the optimized neural network to identify methanol selectively will be assessed.

Keywords: Chemometrics, Imaging, Neural Network, Pattern Recognition
Application Code: Environmental
Methodology Code: Chemometrics
Chemometrics

Application of Orthogonal Signal Correction Algorithms to Noninvasive Blood Glucose Measurements Based on Near-Infrared Spectroscopy

Current approaches to monitor blood glucose levels are based on invasive methods that include finger sticks or continuous glucose monitoring systems that utilize a sensing element implanted in the subcutaneous tissue. Despite advances in these invasive methods, there remains interest in a truly noninvasive blood glucose measurement, particularly for Type I diabetics that have to check their glucose levels multiple times per day. Near-infrared spectroscopy has shown significant promise for use in noninvasive glucose sensing. However, despite many efforts, a practical noninvasive blood glucose measurement based on near-infrared spectroscopy has yet to be realized. One of the principal challenges limiting success is the heterogeneous nature of tissue and the difficulty in establishing a stable background for the spectroscopic measurement. Significant variability in the background tissue matrix can be observed within the same patient, even when attempts are made to measure the same tissue location. Achieving a practical calibration strategy for the measurement requires an effective background correction methodology. In this presentation, orthogonal signal correction (OSC) algorithms are evaluated for use in extracting background spectral features from noninvasive near-infrared spectra collected over multiple days from both human and animal subjects. Several OSC methods have been reported as a way to remove information from spectra that is orthogonal to the analyte of interest, thereby simplifying the spectra to which calibration modeling approaches are subsequently applied. In the work presented here, two OSC methods are compared for use in the noninvasive glucose application, and strategies for their practical implementation are discussed.

This work is funded by Samsung Advanced Institute of Technology.

Keywords: Chemometrics, Data Analysis, Near Infrared, Spectroscopy

Application Code: Other

Methodology Code: Chemometrics
We introduce here the information content (IC), or entropy, which is based on Shannon’s information theory, as a new chemometrics tool for data analysis. This approach is not the same as the mutual information/entropy approaches sometimes used in data processing. Rather it is based on considering a spectrum or set of data points to be a probability distribution according to the following formula:

$$H(x_i) = -\sum p(x_i) \log_2 p(x_i)$$

A discussion of the theory of this technique is presented, which will include IC values of model spectra. We also introduce the concept of the reordered spectrum as a graphical approach for visualizing IC values. IC is shown to be effective in determining the meaningful mass chromatograms to retain in LC-MS, in identifying transitions in depth profiles through a surface/material, for finding trends in images, and in creating PCA-like scores plots of data-sets. That is, we are finding that the IC approach has broad applicability to a number of different data analysis problems.

Keywords: Chemometrics  
Application Code: Material Science  
Methodology Code: Chemometrics
The U.S. EPA Airborne Spectral Photometric Environmental Collection Technology (ASPECT) emergency response program monitors ground sources of volatile organic compounds (VOC) using aircraft-mounted passive Fourier transform infrared (FTIR) spectrometry. The downward-looking entrance optics of the spectrometer collect upwelling ambient infrared radiance from beneath the aircraft along the flight path. When the aircraft flies over an infrared-active species, the spectral signatures of these compounds will be superimposed on the measured infrared background as either absorption or emission features. Though interferograms are collected rapidly (e.g., 70 scans/s), the duration of a scan is such that the scene viewed changes within a scan. In traditional spectral analysis, interferograms are Fourier processed into the frequency domain and the resulting spectra are analyzed for the presence of target spectral features. With this approach, the spectrum being analyzed represents an average radiance over the area covered during the duration of the scan. If the target VOC is only present during a small portion of the scan, the signal is diluted by the surrounding analyte-free area, which could weaken the signal to the point of missed detection. The work presented here describes a procedure by which each interferogram is split into discrete, smaller segments and each segment is treated as an independent measurement. As a feasibility study, we explore whether these small portions of the interferogram can be used to detect ethanol, ammonia, and methanol vapors of varying signal strengths. Both laboratory and field data acquired during aircraft flights are used in this evaluation.
Personal assaults are common and many offenders are aware of potential transfer of DNA and/or fingerprints and thus many actively prevent transfer of such trace evidence. However, many perpetrators overlook the potential transfer of cosmetic components such as shimmer and/or glitter. This project focuses on developing a characterization scheme for transferred shimmer and glitter components collected from a potential suspect, victim, or crime scene for subsequent comparison. The project will aim to distinguish those components and identify features unique to one sub-class or another.

In this study, glitter and shimmer particles were analyzed using scanning electron microscopy-energy dispersive x-ray spectroscopy (SEM-EDS) and Fourier transform infrared (FTIR) spectroscopy. Since shimmer is comprised of metal-oxide coated mica, SEM-EDS was used to determine the composition of the coatings. Similarly, glitter is typically comprised of polyester sheets, painted with FDA approved pigments, and then cut into tiny pieces. Thus, SEM-EDS was used to determine the elemental composition of such pigments. Alternatively, FTIR was used to provide bond-linkage information on the polymeric composition of glitter particles and to differentiate between different types of mica. Muscovite mica and synthetic mica are the most common in the cosmetics industry, both being comprised of different complex aluminosilicate crystals.

Samples were also analyzed using a compound microscope to identify features of area, perimeter, diameter, and thickness. Each feature provided another layer of identification for the glitter and shimmer particles. Statistical analysis was performed via principal component analysis and linear discriminant analysis, allowing for subsequent classification of real world samples.

Keywords: Cosmetic, Elemental Analysis, Forensic Chemistry, FTIR
Application Code: Homeland Security/Forensics
Methodology Code: X-ray Techniques
The analysis of fingerprint samples via pictorial comparisons has been largely accepted by the scientific community as a dependable method of identification. One of the greatest setbacks in fingerprint analysis is that if a matching fingerprint is not saved in a database or if the person of interest is not physically present for comparison, the print is reduced to merely exclusionary evidence, despite being stored in a separate database for future use. The same can be said about DNA. The research presented here investigates the use of two well-known chemical assays in conjunction with the concept of biological sex identification.

The ninhydrin method is the most well-known and widely used. Federal, state, and city crime laboratories have been implementing this technique for almost 50 years. Ninhydrin is a chemical that reacts with amino acids in the fingerprint content to produce Ruhemann’s purple. Here, a modified approach to the traditional ninhydrin method for fingerprint development is combined with an optimized extraction protocol and the concept of determining biological sex from fingerprints.

Despite the success of the ninhydrin method, our group’s intentions are to establish a method where only one metabolite corresponds to one originator attribute. Multianalyte assays that target a larger number of amino acids are not completely reliable because more than one attribute can ultimately effect the output of the assay, thus convoluting the assay’s intentions. To eliminate this possibility, it is important for systems to be restricted to one analyte or a specific combination of analytes that are correlated to the desired originator characteristics. Our first step in this direction was to establish a chemical assay that targeted a small group of amino acids. For this purpose, we chose the Bradford Reagent – traditionally used for protein quantification – because it targets 6 specific amino acids and generates a blue-colored complex.

Keywords: Amino Acids, Bioanalytical, Forensics, Sensors
Application Code: Homeland Security/Forensics
Methodology Code: UV/VIS
A thorough theoretical and experimental study of blood spatter due to a gunshot was conducted with the intent of enhancing bloodstain pattern analysis techniques available to crime scene investigators through the application of fluid dynamics principles.

A fluid of large density accelerating towards one with a smaller density creates an unstable flow situation called the Rayleigh-Taylor instability. This instability is shown to be the process generating a spatter of blood due to a gunshot which allows for the determination of blood droplet sizes and initial velocities. The trajectories of these spattered droplets are then predicted by a model accounting for all relevant forces, namely, the collective effect of droplets interacting with one another in flight which diminishes the drag force of the trailing droplets. Two scenarios, the backward spatter of blood due to a gunshot with a blunt and sharp bullet, were tested and the proposed theoretical model revealed satisfactory agreement to performed experiments.

An analysis of high-speed videos of blood spatter due to a gunshot were conducted to elucidate basic physical mechanisms of the spatter process and thus validate the model. The videos analyzed in this work were of a variety of targets impacted by several types of bullets which caused either forward, backward, or both types of spatter. Using particle image velocimetry and particle analysis software, the investigation revealed that forward spatter results in drops travelling twice as fast compared to backward and that both types of spatter contain drops of roughly the same size. Moreover, it was found that droplets in both forward and backward spatter move with an acceleration due to the aerodynamic wake of preceding droplets spattered initially, at earlier time moments. Therefore, the models in both cases were not only compared experimentally, but were found to agree with high-speed videos of similar phenomena.
Forensic analyses rely on DNA for human identification. However, in the absence of viable DNA, no other evidence type achieves a similar level of statistical probability and power of discrimination. Proteins pose an attractive alternative due to their robustness and derivation from DNA; single nucleotide polymorphisms are often conserved as single amino acid polymorphisms in genetically variant peptides (GVPs). Hair is a common evidence type and, unlike genomic DNA, proteins are notoriously persistent in hair; the medium is well-suited for a proteomics approach for forensic identification.

To validate hair proteome markers for forensic identification, protein variation in hair must be examined. This research aims to assess the protein profiles of single hairs from different body locations and along the length of head hair for three individuals. Protein extracts were concentrated and trypsin-digested for nano-LC-Orbitrap-MS/MS analysis.

Protein profiles were characterized along the length of head hair and protein expression levels were measured. Profiles between head, arm, and pubic hairs from the same individual were also compared. Protein variation was greatest between individuals; the three individuals were differentiated using their protein profiles. GVP markers must be common to the different hair types to differentiate individuals. This research quantifies variation in hair from different locations and identifies marker characteristics for forensic identification.

This work was funded by the LLNL Laboratory-Directed Research and Development Office (16-SI-002) and Livermore Graduate Scholars Program.

This work was performed under the auspices of the U.S. Department of Energy by Lawrence Livermore National Laboratory under Contract DE-AC52-07NA27344. IM# LLNL-ABS-736584.
The trace detection of explosives and related compounds encompasses not only conventional military grade explosives, but the various classes of homemade explosives. Often, explosive devices contain a mixture of organic and inorganic species requiring robust detection schemes targeting both. Traditional trace explosives detection platforms rely on thermal desorption for the vaporization of analytes from collection media prior to chemical analysis. However, for traditional thermal desorption temperatures (<250 °C), many inorganic explosives and oxidizers exhibit low vapor pressures, limiting vaporization and subsequently detection sensitivity. Here, broad spectrum infrared thermal desorption (IRTD) and high temperature resistive Joule heating thermal desorption (JHTD) platforms were developed, each coupled with direct analysis in real time (DART) ionization, and characterized for the mass spectrometric trace detection of inorganic explosives. IRTD employed a filament-based near infrared emitter for the thermal desorption of wipe-collected samples by multi-mode heating – conductive, convective, and radiative. Alternatively, JHTD incorporated the direct passing of current through nichrome wires or metallic meshes laden with analytes of interest. These technologies enabled the generation of discrete and rapid heating ramps, achieving thermal desorption of volatile organic explosives at the initially lower temperatures, while still reaching elevated temperatures to desorb non-volatile inorganic oxidizers. IRTD and JHTD provide unique platforms for the thermal desorption of trace organic and inorganic analytes for the forensic science and security sectors, transportation agencies, and customs and border protection.

The U.S. Department of Homeland Security Science and Technology Directorate sponsored a portion of this work.

Keywords: Forensic Chemistry, Mass Spectrometry, Thermal Desorption, Trace Analysis
Application Code: Homeland Security/Forensics
Methodology Code: Mass Spectrometry
Sweat is a non-invasive, biological fluid that contains amino acids and other low molecular weight compounds. The concentrations of the biochemical content within an individual’s sweat are controlled by hormone metabolism processes that fluctuate regularly based on factors such as age, gender, and activity levels. Since no two individuals will have the same hormone levels at a given time, the concentrations of these sweat components are specific to each individual. Instrumental detection limitations prevent the determination of an individual based on a single analyte. However, monitoring multiple analytes increases the probability of correctly identifying a person based on these metabolic analyte concentrations. Analysis was performed on 50 mimicked sweat samples that were created based on the physiological concentrations of amino acids and small molecules known to be present in sweat. Three of these compounds were studied using three separate single-analyte enzymatic assays and ultraviolet-visible spectrophotometry to determine the concentrations of these compounds within an individual’s sweat. Additionally, a collection and extraction method was successfully developed to collect authentic sweat samples from volunteers. The application of sweat collection decreases the sampling error compared to fingerprint collection methods and increases the amount of sweat collected to aid in analysis of small molecules at low concentrations. The forensic science community will benefit from a novel sweat analysis approach capable of differentiating metabolic compounds inherent in each individual’s sweat. The combination of these levels demonstrates the potential of sweat analysis for biochemical identification purposes to increase security measures by serving as a locking mechanism for electronic devices. Future research into sweat analysis can lead to an alternative to DNA identification processes via the bio-affinity detection of small molecules in sweat.

Keywords: Amino Acids, Biosensors, Enzyme Assays, UV-VIS Absorbance/Luminescence
Application Code: Homeland Security/Forensics
Methodology Code: UV/VIS
Homeland Security/Forensics - Technological Advances

Characterization of VOCs Emitted from Pathogenic Bacteria Using SPME-GC-MS: Towards Non-Invasive Breath Diagnostics for Biosecurity

Medical services need rapid, non-invasive diagnostics for pathogen infections. Trace volatile organic compounds (VOCs) in exhaled breath offer candidate indicators of human physiological, metabolic, and disease states. Detection and interpretation of VOCs in breath remains an underutilized strategy but offers potential to yield an information-rich matrix of biomarkers. An application for biosecurity proposes to diagnose victims of biological attacks, requiring differentiation of the chemical signatures from exhaled breath and biological agents. This work characterizes VOCs released from [i]Francisella tularensis[/i] novicida (strain U112) and [i]Bacillus anthracis[/i] Sterne, surrogates for potential biowarfare agents.

[i]F.t. [/i] novicida was cultured in modified Mueller-Hinton media and [i]B.a.[/i] Sterne was cultured in Brain-Heart Infusion media. Emitted VOCs were sampled using solid-phase microextraction (SPME) at multiple time points and characterized using gas chromatography-mass spectrometry (GC-MS). Compound identification was accomplished through comparison of chromatographically-deconvoluted experimental spectra to library spectra (NIST14 match 80%).

Differentiation of [i]F.t. [/i] and [i]B.a.[/i] was accomplished through qualitative and quantitative comparisons of the species-specific VOC fingerprints, reproducibly derived from multiple biological replicates. Biological functions were attributed to validated biomarkers, e.g. odd-carbon numbered aliphatic methyl-ketones identified in [i]F.t.[/i] novicida were attributed to metabolites formed during oxidation of fatty acids. Detection of VOC biomarkers emitted from [i]F.t., B.a.[/i], and other bacteria through SPME-GC-MS has the potential for rapid and non-invasive identification and monitoring of bioterrorism-relevant pathogens.

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Antibody-drug conjugates (ADCs) are hybrid therapeutic modalities designed to improve cancer treatment through precision killing with fewer side effects. They are typically constructed from a potent cytotoxic drug covalently conjugated to a specific monoclonal antibody through a carefully designed linker. Due to the presence of multiple components, ADC manufacturing is a complex process and impurities can be introduced at any step throughout the process. Effectively monitoring and controlling these impurities are required to assure product quality and patient safety, as well as to demonstrate regulatory compliance. However, this wide range of potential impurities, together with their distinct physicochemical prosperities, such as polarity, charge state, and lack of UV absorption, make monitoring of residual impurities in ADCs quite challenging. In this work, a hydrophilic interaction chromatography (HILIC) coupled with charged aerosol detector (CAD) method was developed for multiplexed detection of residual process impurities in ADCs. Eight reagents commonly used in ADC manufacturing, including antimicrobial agents added in cell culture, reducing/oxidizing/capping agents needed in conjugation process, and stabilizing agent introduced in formulation development, etc. were separated within 20 min. A silica-based mixed-mode column with cation-exchange, anion-exchange, and reversed-phase mode, operated under HILIC condition, was used and mobile phases were optimized regarding buffer ions, ionic strength, pH, etc. This method has been successfully applied to detect residual impurities in ADCs with excellent selectivity, sensitivity, and reproducibility. It presents a simple, fast, and generic approach for simultaneously monitoring multiple process residual impurities to speed up ADC development.
Forced degradation studies are typically performed to understand the degradation pathway of pharmaceuticals. Mass balance correlates the measured loss of a parent drug to the measured increase in the amount of degradation products, however, given the range of impurities and their chemical and physical properties, mass balance studies can be challenging. One of the specific challenges includes determining the response factor of an impurity relative to the active pharmaceutical ingredient (API). Incorrectly identifying the relative response factors (RRFs) could lead to over or under quantification of the impurity, which can in turn lead to mass imbalance. However, to determine the RRF of an impurity by UV a pure standard is typically required. This poses a challenge for those impurities that may be unknown or for which there are no standards readily available. Isolation or collection of these impurities provides an opportunity to further characterize the impurities.

In these studies, a dual detection system consisting of a photodiode array (PDA) and a mass detector in combination with small scale fraction collector will be used to analyze a stressed drug substance. Fraction collection will be performed on an analytical scale for numerous impurities in the sample, including low level species. Using these collected samples, calibration curves will be acquired. The relative response factors (RRFs) of these impurities will then be determined by established methodologies, specifically the ratio of the slope of both the API and impurity standard curves. For comparison, the RRFs will also be determined using purchased standards, when applicable, to verify the approach. The subsequent RRFs will then be used to calculate mass balance for the stressed study.
Trihexyphenidyl is an active pharmaceutical ingredient (API) with clinical value in the treatment of Parkinson’s disease. Previous to this work, the USP Trihexyphenidyl Hydrochloride Tablets monograph did not carry an Organic impurities (OI) procedure. In an effort to modernize the monograph, a single stability-indicating HPLC method was developed and validated for OI and Assay. Chromatographic separation was achieved on a core-shell column (2.1 x 100 mm, 2.6 μm), operating under a gradient flow of mobile phases comprised of potassium phosphate buffer and 0.05% phosphoric acid in acetonitrile with detection at 210 nm. Stability-indicating criteria were established through forced degradation studies of the API. The oxidative stressed API showed degradation with the formation of three degradants, well resolved from the API peak and from each other. PDA analyses of the API peak in all stressed samples were determined to be spectrally pure, indicating a lack of co-elution. Robustness study demonstrated that minor variation to chromatographic parameters did not lead to system suitability failure. The OI procedure validation was carried out in the range of 0.1% - 1.5% of the sample nominal concentration of 1.0 mg/mL, and validated for specificity, linearity, accuracy, precision, ruggedness, and solution stability. The method was also validated for assay in the analytical range of 0.07-0.13 mg/mL with a sample nominal concentration of 0.1 mg/mL. The method was established to be applicable to trihexyphenidyl hydrochloride tablets from multiple manufacturers. A stability-indicating HPLC method was developed and validated for compendial use in purity and strength testing of trihexyphenidyl hydrochloride tablets.
The Pharmaceutical Industry has a lot of interest in using peptides as therapeutic agents. Synthetic peptide technologies have developed sufficiently to allow for large scale manufacturing. These crude synthetic peptide mixtures contain many closely related components and it is very difficult for a chromatographic method to separate the desired component from the other components. The ability of a chromatographic method to separate different components is called selectivity. The easiest way to change chromatographic selectivity is to change the stationary phase. The proper stationary phase selection will easily separate the desired component from components that are not closely related. However, the separation of closely related components can be very difficult to resolve from each other. Fortunately, the very nature of peptides allows for significant selectivity differences when the eluent changed in pH or the type of organic solvent.

The work presented here will outline a systematic approach to develop HPLC methodology for synthetic peptides. This approach is a five-step process and is designed to yield suitable methodology for HPLC analysis. Each step is described as a procedure and the function of each step is discussed. The five steps include; identification of the peptide properties, column identification, pH selection, organic solvent determination and final optimization. The five-step process will be demonstrated with a commercially significant synthetic peptide.
Superficially Porous Particles (SPPs) of diameters smaller than 3 microns were first introduced as chromatographic packings about 12 years ago. Since that time, these materials have been called by several different names, including core-shell, solid-core, partially porous, and pellicular. Due to the unique particle design, columns packed with silica particles consisting of a thin porous shell and solid silica core are now widely used in the pharmaceutical industry since faster separations are enabled without deleterious effects (loss of resolution/broad peaks). Although the particle technology has been around for a considerable time and its physical advantages suitably utilized, there remains a need for unique column selectivities to separate the increasingly wide variety of sample mixtures. This paper will present examples of new and different phases, including a new polar endcapped C18 phase for high-aqueous mobile phase separations. C18 columns with accurately-controlled amount of stationary phase polarity complement classic C18 columns by retaining and separating polar compounds better and by resisting the tendency for pure hydrophobic phases to lose retention and peak shape under high-aqueous mobile phase conditions. Examples of separations ranging from highly polar to extremely hydrophobic compounds will be shown and the advantages of using particular bonded phases on SPP silica packing materials will be discussed.
This presentation will discuss method challenges and mitigation strategies associated with a HPLC impurity method used for a pediatric oral solution formulation. The proposed oral solution formulation contains high concentrations of surfactants (tween-80 and sodium dodecyl sulfate) and polymer (PEG-400) designed to enhance the overall formulation solubility. Additionally, propylene glycol, glycerin (preservatives) and orange flavor were added to increase patient palatability. The presence of these functional excipients posed a number of analytical challenges such as a noisy baseline, a hump in the baseline in close proximity to major degradation product peaks, variability in placebo related peaks and several unknown peaks with unknown origin observed during product stability studies. Attempts to optimize the existing HPLC method to improve the baseline and selectively remove placebo related interferences will be discussed. Development and optimization of a new UPLC method using DryLab 4 and Fusion-AE DOE software to identify and test critical analytical method factors for the proposed commercial formulation will be presented. A risk assessment was conducted to evaluate the impact of analytical method risks on drug product quality, safety and efficacy and mitigation strategies were put together to minimize the impact and any residual risks that could not be mitigated were communicated to appropriate stakeholders.

Keywords: Analysis, HPLC, Method Development, Pharmaceutical
Application Code: Pharmaceutical
Methodology Code: Liquid Chromatography
Sodium thiosulfate is an active pharmaceutical ingredient (API) approved by the United States Food and Drug Administration. Dosing sequentially with sodium nitrite, sodium thiosulfate injection solution is used for the treatment of acute cyanide poisoning that is judged to be life-threatening. Sodium thiosulfate is also being tested as an extravasation antidote for cancer treatment to lessen the side effects of cisplatin (a chemotherapy agent). As part of the United States Pharmacopeia (USP) modernization effort, an ion chromatography (IC) method has been proposed to replace existing titration-based assays in the Sodium Thiosulfate and Sodium Thiosulfate Injection monographs. In addition, another IC method has also been proposed for determining chloride, sulfate, and sulfite impurities in Sodium Thiosulfate and sulfate and sulfite impurities in Sodium Thiosulfate Injection. Here, both methods are evaluated following the guidelines outlined in USP General Chapter <1225>, Validation of Compendial Methods. Briefly, we followed the monograph instructions for each method and then made deliberate variations in the IC method parameters (e.g. mobile phase concentration, column temperature, etc.) to test robustness. We found that the Sodium Thiosulfate assay method is linear (r^2=0.999) over the established analytical range of 0.2-150 mg/L. The method is sensitive (LOQ at 0.2mg/L), accurate (intraday and inter-day accuracy 99–106%), precise (precision <0.3%), and specific for Sodium Thiosulfate assay. The Sodium Thiosulfate impurity method is linear over the established analytical range for impurities (chloride: 0.04-2.00 mg/L r^2=1.00, sulfite: 0.1-5.0 mg/L, r^2=0.9998 and sulfate: 0.5-10.0 mg/L r^2=1.00). We found the method was sensitive (LOQ of chloride =0.01, sulfite =0.2, and sulfate = 0.05 mg/L), and accurate (recovery 85–108%). We repeated these tests with a second column and found the method was robust to both changes in chromatography parameters and column change.
Novel LC-UV method was developed and validated for estimation of carvacrol release from carvacrol loaded nanobeads. Carvacrol is used as anti-infective agent. Carvacrol nanobeads are proposed for topical application on skin. In-vitro drug release of carvacrol from topical formulation is performed in a vertical diffusion cell (Franz diffusion cell). Infective skin’s pH is 7.15-8.9, so drug release study was performed at pH 7.2 and pH 8. HPLC method was optimized by Design of Experiment Approach (DOE) approach using Design of Expert software. Box behnken design was applied with three factors such as mobile phase ratio, pH and flow rate at three levels. Four responses were studied on 17 trials and final optimised LC method was selected based on design space (Figure 1). Mobile phase finalised was Tetrahydrofuran: Acetonitrile: Water: Triethylamine (70: 18: 12: 1 %v/v) having pH 6.5 which was adjusted with glacial acetic acid. Chromatographic column used was C18, 5µm particle size. Carvacrol was detected at 275nm and 1 ml/min flow rate. LC method was validated as per ICH Q2 (R1) guideline. Linearity was found in specific range with correlation coefficient more than 0.999(Figure 2). Method was proved to be precise, specific, accurate and robust. Drug release at pH 7.2 was found to be 82.87 which is less than 90.17 in phosphate buffer pH 8 at 24 hours (Figure 3).

Keywords: Chromatography, HPLC, Method Development, Pharmaceutical
Application Code: Pharmaceutical
Methodology Code: Liquid Chromatography
Mass Spectrometry - Omics, Food Identification/Science and Homeland Security

Single-Injection Method for Quantitative and Qualitative Analysis of Amino Acids Using IROA with UHPLC-HRMS

In classical targeted quantification assays, the strategy has been restricted to relative quantification based on the relation of biological studies to purchased isotopic standards. This method can be restrictive due to its low metabolite coverage and high cost, where analytes of interest require individual corresponding standards. Isotopic ratio outlier analysis (IROA) allows for the generation of isotopically labeled control standards of an entire metabolome. This is accomplished by incorporating 95% 13C IROA media to produce a unique isotopic pattern, facilitating both identification of analytes and quantitation. This allows for a more biologically relevant method and improves the confidence of metabolomics analyses due to a readily identifiable isotopic pattern.

95% 13C labeled IROA yeast extract, *Saccharomyces cerevisiae*, was used as an internal standard and added to pooled plasma. A calibration curve was generated using 5% 13C IROA labeled yeast at a concentration range of 0.01 mg/mL to 20 mg/mL. A total of 40 amino acids were targeted and validated against standards to confirm m/z and concentrations of each analyte. Data were collected on a Thermo Q-Exactive mass spectrometer that was operated in full scan mode. Chromatographic separation was achieved using reverse-phase high performance liquid chromatography and hydrophilic interaction chromatography.

The purpose of this study was to develop a highly reproducible method for quantification without purchasing individual internal standards. Instead, quantification was accomplished by creating a calibration curve utilizing the 5% labeled IROA yeast. By labeling 5% of the carbon sources in the yeast with 13C, the metabolites produce a highly identifiable spectrum, where there familiar M+1 peak is increased from approximately 1% per carbon in a molecule at natural abundance to approximately 5%.

Keywords: Amino Acids, HPLC, Isotope Ratio MS, Mass Spectrometry

Application Code: Genomics, Proteomics and Other ‘Omics

Methodology Code: Mass Spectrometry
Immunoglobulin M (IgM) is the first Immunoglobulin generated in the human body in response to antigens. It is the largest antibody in the human circulatory system with molecular weight close to 1 million Daltons (Da), which makes it difficult to be characterized by analytical methods. In this study, we evaluated the possibility of using mass spectrometry platforms to fully characterize IgM samples. Various species IgM standards were applied in the testing experiments. The intact IgM samples were analyzed by Shimadzu AXIMA MALDI-TOF-MS with CovalX MegaTOF™. After optimizing the instrument parameters, data processing methods and sample preparation methods, the average molecular weights of intact IgM standards were determined as between 900 kDa and 1050 kDa, with CV 0.5% (n=36). For reduced IgM characterization, samples were first denatured in urea or Guanidine-HCl, and then reduced by TCEP or DTT. After 30 min incubation, both TCEP and DTT were able to achieve near complete IgM reduction. The heavy chains and light chains were detected by ESI-MS and/or MALDI-TOF-MS. For IgM glycosylation analysis, 5 different deglycosylation enzymes were used to deglycosylate both intact and reduced IgM samples. Extensive N-glycosylation was observed on the heavy chains of IgM samples by MALDI-TOF-MS and ESI-MS. In summary, we have successfully used MALDI-TOF-MS and ESI-MS platforms to characterize the intact, reduced, and deglycosylated IgM.
Mass Spectrometry - Omics, Food Identification/Science and Homeland Security

Certification of Marine Toxins by Quantitative NMR and Isotope Dilution MS at the Highest Metrological Level

Since the presence of marine toxins in shell fish and sea food in general is an emerging problem, fast and sensitive LC-MS methods were established very recently for food testing. Therefore, the access to well characterized reference materials for a precise and accurate quantitation of these different toxins is now a crucial need of the testing laboratories. Unfortunately, the isolation, synthesis and purification of these compounds or their stable isotope labeled analogs is very challenging and lot sizes are typically in the range of few mg. In order to achieve certification of such small batches under ISO/IEC 17025 and ISO Guide 34 double accreditation at the highest metrological level, a combined setup of qNMR and Isotope Dilution MS was successfully established. In a first step, the concentration of a dissolved toxin is determined by a series of 1H-HP®-qNMR measurements. The received certified mass fraction is subsequently applied in an LC-IDMS experiment that results in a certified concentration for the stable isotope labeled analog. IDMS experiments are also carried out to determine the homogeneity and stability contribution to the overall uncertainty. The setup was validated by the defined and well characterized system caffeine-13C3-caffeine. An intra-laboratory comparison (3 persons, 3 days, 2 balances, 2 different reference solutions) of independently prepared caffeine/13C3-caffeine standards and sample solutions showed an overall variation +0.2/-0.2 µg/g of the measured concentrations. After demonstration of the robustness of the method, several saxitoxin derivatives were analyzed and certified using the same methodology.

Keywords: NMR, Quantitative
Application Code: Food Identification
Methodology Code: Mass Spectrometry
Coffee and tea are very popular drinks for people in modern society. There have been many papers reporting positive and negative effects of coffee and/or tea based on a couple of major components. However, coffee and tea have multiple components and their effects have to be collectively considered. For more complete understanding, the chemicals comprising the coffee and tea have to be accurately identified. Ultrahigh resolution mass spectrometry (UHR-MS) is a powerful tool to study complex mixture at the molecular level. UHR-MS have been successfully applied to study organic mixtures in food. In fact, there has been a couple of reports on analysis of coffee and tea by use of (-) electrospray ionization UHR-MS. However, considering the selectiveness of ionization techniques used for MS, it is reasonable to expect that employing various ionization techniques for a given sample is beneficial. Therefore, in this study, atmospheric pressure chemical ionization (APCI) and laser desorption ionization (LDI) coupled to UHR-MS have been applied to identify chemical compounds in coffee and tea extracts. Our preliminary result shows that (-) LDI UHR-MS can be successfully applied to study coffee. The coffee roasted at different temperatures were extracted and the extracts was analyzed by use of (-) LDI UHR MS. Quinic and caffeic acids were found. Especially, a series of compounds separated by [caffeic acid – H2O] were identified. In addition, a number of peaks were identified from the coffee beans roasted at 195 degree. Further research is undertaken to identify the chemicals.

**Keywords:** Analysis, Food Identification, Food Science, Mass Spectrometry

**Application Code:** Food Identification

**Methodology Code:** Mass Spectrometry
We present a novel analytical setup based on Proton-Transfer-Reaction – Time-of-Flight Mass Spectrometer (PTR-TOFMS) with a fastGC pre-separation and coupled to an auto-sampler, that allows for comprehensive high throughput aroma profiling.

PTR-MS is one of the most sensitive methods for real-time analysis of VOCs (Volatile Organic Compounds). Coupling the soft PTR ionization with a TOF mass spectrometer allows the analysis of a complete VOC spectrum in real-time (<100ms), which is essential to study dynamic processes or can also be exploited to analyze discrete samples at high throughput. For the latter, the addition of a fast gas-chromatographic pre-separation (fastGC) offers several important advantages: The high TOF mass resolution is supplemented by the GC retention-time which yields complementary information on the compound. In addition, this greatly facilitates the separation of isomeric compounds, which is essential for complex samples. A surplus is the separation of the inherently high concentrations, which would otherwise compromise the quantification of other trace VOCs. This problem is typically found in the head-space of alcoholic beverages with an inherently high ethanol concentration.

We now complement a fastGC-PTR-TOF by an auto-sampler to allow for automated, comprehensive, high-throughput analysis. We apply this to aroma profiling of wine samples: The analysis of one sample takes less than 2 minutes and more than 300 distinct VOC related signals, each including exact mass information (chemical composition), retention time (chemical properties), and signal intensity (concentration). The amount and quality of measured information in such a short amount of time is outstanding for this application.

Keywords: Beverage, Chemical Ionization MS, Trace Analysis, Volatile Organic Compounds
Application Code: Food Science
Methodology Code: Mass Spectrometry
High-field asymmetric-waveform ion mobility spectrometry (FAIMS) is an emerging technology that has great potential for targeted applications. FAIMS utilizes an alternating asymmetric electric field to separate ions by their different mobilities at high- and low-fields as they travel through the separation space. When coupled to mass spectrometry, FAIMS enhances the separation of analytes from other interfering compounds with little to no increase in analysis time. Several strategies have been investigated to improve the FAIMS separation for small isomeric molecules. These strategies include adding solvent vapor to the FAIMS cell and using various cations to form ion complexes that enhance ion mobility differences between isomers.

In this work, we utilize these strategies to separate isomers for two groups of drugs: anabolic androgenic steroids and opioids. The use of anabolic steroids as performance enhancing substances is prohibited in sporting events by the World Anti-Doping Agency, leading some athletes to use designer steroids to evade detection. Opioid addiction is an escalating problem that is compounded by the introduction of synthetic opiate analogues such as fentanyl. Screening methods for both of these compound classes are being challenged by the availability of synthetically manufactured analogues, including isomers of existing substances.

Solvent vapor addition has been demonstrated to dramatically improve analyte separation in FAIMS-MS. When dry nitrogen gas is used in the FAIMS cell, the FAIMS peaks for the [M+H]+ ions of morphine and norcodeine cannot be resolved (m/z 286). However, when a mixture of nitrogen gas and acetonitrile vapor is used, morphine is baseline resolved from norcodeine. Cation addition can produce ion adducts for specific isomers that result in unique FAIMS peaks. For example, the [2M+K]+ ion for testosterone (m/z 615) results in two peaks, with one peak that is fully separated from the single peak of epitestosterone.

Keywords:  Drugs, Forensics, Instrumentation, Mass Spectrometry
Application Code:  Homeland Security/Forensics
Methodology Code:  Mass Spectrometry
Applying a High Quality and Comprehensive Tandem Mass Spectral Library to Human Metabolite Identification

Mass spectral library searching is becoming a fast and accurate data analysis technique for identifying metabolites in complex mixtures from LC/MS/MS data. We have been extending a tandem mass spectral library with high quality reference MS2 spectra of various precursor types, including ions produced in-source, isotopic precursors, and MSn spectra. We also examined and applied this comprehensive library to human metabolite identification. Mass spectra of each authentic compound were acquired with an Orbitrap Elite instrument (IT, FT-IT, and HCD). All the precursors were identified based on the chemical structure. Then the spectra with the same precursor were clustered based on the fragmentations. Furthermore, a consensus spectrum at the same instrument condition was generated for the library.

Human plasma and urine samples were analyzed on reverse phase LC/MS/MS (Orbitrap Fusion Lumos). The extended tandem mass spectral library contains >650,000 spectra from >15,000 compounds. Of these, >3,500 are human metabolites. These spectra include >130,000 high resolution MS2 spectra from >10,700 in-source fragment ions, >18,000 spectra of isotopic precursors for 555 compounds with Cl, Br or Sn, and >97,000 low resolution MS3 and MS4 spectra. The library was used to identify metabolites in human serum and urine samples. Some metabolites were identified with not only [M+H]+ and [M-H]-, but also [M+Na]+, [M+2H]2+, etc. The ions produced in-source by various neutral losses (e.g. [M+H-NH3]+ from tryptophan) were also identified in 48% of the identified metabolites. Although the structures of the in-source fragments can be easily deduced from the original compounds, these sub-structures may not exist in the real sample. The spectra of in-source fragments in the library can not only identify common fragmentation artifacts and high duplicate “unknown” spectra, they can also confirm metabolite identification and help identify metabolites that are not in the library.

Keywords: Data Analysis, Informatics, Tandem Mass Spec, Metabolomics
Application Code: High-Throughput Chemical Analysis
Methodology Code: Mass Spectrometry
Thermal desorption direct analysis in real time - time of flight mass spectrometry (TD-DART-TOFMS) is a novel technique in forensic analysis in which an independent thermal desorption unit is coupled to a DART-TOFMS. The sample is thermally desorbed in an enclosed chamber by the thermal desorption unit, allowing for more sensitive analysis compared to traditional DART-TOFMS.

In this study, pre- and post-burn smokeless powder extracts were analyzed by traditional DART-TOFMS and TD-DART-TOFMS. Unburned or partially burned smokeless powder particles and burned smokeless powder residues may be recovered following an explosive event, such as discharge of a firearm or an improvised explosive device (IED). Therefore, unburned and burned samples were analyzed to simulate possible real world evidence.

Smokeless powders were analyzed for the presence of several organic compounds of interest, including: nitroglycerin, diphenylamine, ethyl centralite, dinitrotoluene, phthalates, and others. Extracts of unburned, i.e. pre-burn, smokeless powders and post-burn smokeless powder residues were prepared following a simple extraction procedure using dichloromethane. While extraction is not necessary for DART-MS analysis, extracts were utilized to minimize sample variability, allowing for simple, direct comparison between the methods. DART-TOFMS and TD-DART-TOFMS results were compared using multivariate statistical approaches. Classification schemes were developed for pre- and post-burn smokeless powder extracts. Thermally desorbed samples were used as an external test set to test classification within the traditional DART-TOFMS models as part of the validation of the thermal desorption technique for smokeless powder analysis.

Keywords: Forensic Chemistry, Mass Spectrometry, Thermal Desorption, Validation
Application Code: Homeland Security/Forensics
Methodology Code: Mass Spectrometry
Sample Preparation and Microextraction

Analysis of Bisphenol A in Foods using Solid Phase Microextraction with an Overcoated Fiber

Bisphenol A (BPA) is a common component of food packing materials, including the linings of metal cans. While the safety of exposure to BPA is still under debate, public concern has increased regarding ingestion of this compound via packaged foods. Various methods have been used to analyze BPA in food, many using liquid/liquid extraction and/or solid phase extraction. In this work, solid phase microextraction (SPME) using an overcoated fiber was used for the immersion extraction of BPA from various packaged foods followed by GC/MS/MS analysis. The optimized SPME method was highly sensitive, allowing for low ppb level detection of BPA. The fiber used for the application consisted of divinylbenzene (DVB) overcoated with polydimethylsiloxane (PDMS). This overcoating allowed for direct immersion of the fiber into food matrices by protecting the DVB coating from sugars and fats present in the samples. A wash step after extraction was used to remove excess sample on the surface of the fiber, thus prolonging fiber life. Aspects of method development using the overcoated fiber, along with accuracy and reproducibility from several food matrices will be presented.

Keywords: Food Contaminants, GC-MS, Sample Preparation, SPME
Application Code: Food Safety
Methodology Code: Sampling and Sample Preparation
Sample Preparation and Microextraction

New Advances in Automated Sample Preparation for Ion Chromatography

Based on recent surveys, the automation of sample prep processes continues to dominate the wish list of analytical chemists. Offline sample prep processes are often criticized for adding cost which includes both the cost of the devices and also the labor required to treat samples offline. Automation can improve the reproducibility of an analysis by tightly controlling processes including timing, flow rate, volumes delivered, mixing parameters. Automation may also offer lower costs compared to offline processes by reducing labor and support items such as disposable syringes, and by allowing the use of instrumentation during extended hours etc. In many automated systems though there can be considerable cost associated with the instrumentation itself, complexity of setting up the automated methods, maintenance, to list a few of the concerns.

In ion chromatography, sample preparation is often focused on matrix elimination of interfering non-analyte species including species that interfere with separation and/or detection, or foul the system. The workhorse technique is that of matrix elimination using offline cartridges such as OnGuard although some inline techniques are useful using multiple use cartridges such as InGuard. Now we have developed simple-to-use vial caps containing filters that are loaded with several types of stationary phases. As the sample is flowed thru Guardcap H, Na or Hydrophilic Reversed Phase (HRP) filters by the unique action of the AS-DV or AS40 autosampler, processes including matrix elimination of metals or surfactants with or without changing the pH of the sample and neutralization of bases are easily accomplished. Labor is minimized and since each vial cap is used on one sample there is no concern about contamination or carryover from previous samples. In this paper we will report on two new Guardcap chemistries, Na and HRP, show the features of this concept and also key applications for three chemistries.

Keywords: Automation, Instrumentation, Ion Chromatography, Sample Preparation
Application Code: Environmental
Methodology Code: Sampling and Sample Preparation
Perfluorinated compounds (PFCs) are of increasing concern as they are detected in environmental and biological matrices. Originally thought to be inert compounds, they are long-lived and may cause tumors and endocrine effects. Continued exposure to these persistent and bio accumulative compounds may be especially hazardous. They are of concern in the US as a result of UCMR-3 and detection in drinking water. They are also of concern to the DOD and many military bases are contaminated as a result of fire-fighting foam. In Europe there is concern as a result of the Stockholm Convention (Annex B, 2009) and PFCs are classified as extremely persistent environmental contaminants.

This paper will discuss some of the extended lists of compounds including perfluoroalkyl carboxylates/sulfonates/sulfonamides, precursor compounds and derivatives. The challenges of extracting a wide range of compounds with different functional groups and chemical properties will be explored and advances in advances in SPE sorbents and formats will be discussed. Data will demonstrate the progress to-date in new technology for SPE and automation.

Keywords: Environmental Analysis, Mass Spectrometry, Solid Phase Extraction
Application Code: Environmental
Methodology Code: Sampling and Sample Preparation
Accelerated solvent extraction (ASE) is a high-temperature and high-pressure extraction technique that is widely used in environmental, chemical and food analysis. Extractions at high temperatures and pressures allow faster extraction of analytes relative to conventional solid-liquid or liquid-liquid based extraction techniques. Moreover, parallel extraction offer the advantage of faster operation. Gas assisted extraction is a method of extracting an analyte from a sample that uses a mixture of a liquid solvent and a gas through the sample container.

In this presentation we discuss a new parallel extraction protocol for pursuing extractions in gas assisted extraction mode and standard ASE mode. We present here performance data of the new method for analyte extraction from a variety of food and environmental samples. We will also show performance data comparing the performance of the new method with the standard extraction method in terms of analyte recovery. The key benefits of the new parallel extraction protocol method are faster and lower solvent usage coupled with a reduction in extraction time. The new methods presented here enhance the utility of pressurized solvent extraction.

Keywords: Accelerated Solvent Extraction, Environmental, Extraction, Sample Preparation
Application Code: Environmental
Methodology Code: Sampling and Sample Preparation
Comprehensive two-dimensional gas and liquid chromatography are among the most powerful analytical separation techniques. In both of them fractions of the first column effluent are repeatedly collected and then periodically reinjected into a second column of different selectivity. This is accomplished through the use of special interfaces between the two columns.

The success of GC×GC is due in large part to its relative simplicity. While the modulation process in LC×LC is typically more straightforward, the separation in the second dimension is much more challenging because of the need for very rapid gradients and quick re-equilibration when completely orthogonal separation mechanisms are used. In GC×GC, second dimension separations are carried out under practically isothermal conditions, which makes the process simple, but the performance of the technique often suffers because of that. By using the best approaches from GC×GC and LC×LC, both techniques can be simplified and/or their performance improved. In GC×GC, a significant improvement in peak capacity can be realized by using fast temperature programming in the second dimension. In LC×LC, very good separations can be realized by using partially correlated separation mechanisms and parallel gradients. Examples of both approaches will be presented.

Keywords: Chromatography, Gas Chromatography, Liquid Chromatography
Application Code: Other
Methodology Code: Separation Sciences
The trend of drug discovery shifted from small molecule drugs to biomedicines. Eight of the ten top-selling drugs in 2015 in the world are biopharmaceuticals such as insulin, and monoclonal antibody drugs, and the market of biopharmaceuticals is further growing. However, pharmaceutical prices of monoclonal antibody drugs are extremely hard to sustain for patients. Such prices are also due to the enormous costs for antibody purification, because the purification of monoclonal antibodies is commonly performed with expensive and short-lived Protein A columns. The development of a purification method for proteins, such as antibodies, under mild conditions and with sustainable costs is strongly needed.

Therefore, we developed a purification method for proteins such as monoclonal antibodies with a low-cost column. We prepared poly(N-isopropylacrylamide) (PNIPAAm)-based thermo-responsive hydrogels with aminopropyl silica (40–64 μm), which are relatively inexpensive compared with the classified silica beads of smaller size that are applied to stationary phases for HPLC. PNIPAAm exhibits temperature-responsive phase transitions (hydrophilic/hydrophobic). This temperature-responsive SPE system is able to control the elution behavior by only changing the temperature of an aqueous eluent with neutral pH.

Lysozyme was separated from an ovalbumin mixture without the loss of bioactivity (99.7% of activity compared with the activity before the separation step). Monoclonal antibody, rituximab was also purified from BSA, the major impurity without observation of hydrolysis and aggregation of rituximab. These results show that temperature-responsive polymer-based SPE can be applied to biomedical purifications with limited costs. The development of a purification method employing inexpensive materials contributes to reduce production costs for expensive biomedicine reagents, such as monoclonal antibodies.

Keywords: Biomedical, Polymers & Plastics, Separation Sciences, Solid Phase Extraction
Application Code: Biomedical
Methodology Code: Separation Sciences
Enhanced Extraction of Bioactive Components from [i]Aloe Barbadensis Miller[/i] Using Environmental Friendly Deep Eutectic Solvents

Deep eutectic solvents (DESs) are emerging green solvent, which may be used for highly efficient extraction of natural products from plants biomass. DESs consist of cheap and simple compounds with relatively high safety profile and extracts may directly be used in pharmaceutical, food, cosmetics etc. In present study, four DESs were prepared by different combination of choline chloride with hydrogen bond donors such as glycerol, ethylene glycol, tartaric acid and oxalic acid. These solvents were applied for the extraction of bioactive component from [i]Aloe barbadensis Miller[/i] by simple maceration, ultrasound assisted and microwave assisted methods. The extraction yield was high for DESs such as ChCl:Gly (203mg/g), ChCl:EG (261mg/g), ChCl:TA (301mg/g), ChCl:OA (325mg/g) and aq.MeOH (150mg/g). Antioxidant activity (10.9-81.3%) was assessed by DPPH free radical scavenging assay. Bioactive ingredients were quantified by total phenolics (72.5-147.5 mg GAE/g) and total flavonoids (35.1-142.3 mg QE/g). The HPLC profile of bioactive ingredients confirmed the presence of quercetin, gallic acid, 4-hydroxy-3-methoxy benzoic acid, chlorogenic acid, syringic acid, m-coumaric acid and cinnamic acid. The antibacterial assay of extracts was evaluated by established biological protocols. In the antibacterial activity the zone of inhibition against bacterial strains of [i]E. coli[/i] and [i]Bacillus subtilis[/i] were in the range of 10-25mm and 9-22mm, respectively. Results of various assays were analyzed statistically by applying regression analysis. Microwave-assisted-extraction exhibited higher extraction yield and shorten extraction time than ultrasound assisted, heat stirring and maceration. DESs extracts of [i]Aloe barbadensis Miller[/i] exhibited potential antioxidant and antibacterial properties. DESs can act as designer solvents and current study suggests their use as safe extraction media for bioactive components used in cosmetics, food flavour, pharmaceutical etc.

Keywords: Accelerated Solvent Extraction, Liquid Chromatography, Natural Products, Separation Sciences
Application Code: Food Science
Methodology Code: Separation Sciences
Separating proteins and antibodies is a unique analytical challenge owing itself to the complexity of the analytes. This complexity is a result of the myriad number of modifications to these analytes in a given sample including, but not limited to, phosphorylation, methylation, and glycosylation. Both heterogeneity and non-specific binding to the silica surface often result in extensive peak broadening and tailing. This seminar will focus on several different chromatographic strategies that the analytical scientist can employ to resolve and quantitate various biomacromolecules, including mAbs and ADCs. Selected aspects of size-exclusion, hydrophobic interaction, ion-exchange, hydrophilic interaction, and reversed-phase chromatography will be discussed. Finally, some aspects of method development will be presented to aid in the development of robust and reproducible analytical chromatographic schemes for the separations of protein and antibody mixtures.

Keywords: Bioanalytical, HPLC Columns, Liquid Chromatography, Protein
Application Code: Bioanalytical
Methodology Code: Separation Sciences
Electronic cigarettes (e-cigs) are commonly used as an alternative to traditional smoking. New generations of e-cigs are customizable, potentially leading to different negative health repercussions for the user. Metal composition of the coils used in atomizers has been demonstrated to change as the e-cig is activated, showing significant losses of iron, chromium and nickel. The objective of this research was to evaluate whether metal particulates are aerosolized and inhaled by the user, or aerosolized and condensed on the inside of the mouthpiece of the e-cig atomizer.

Coils were built using two types of wire, 30 AWG Kanthal and 30 AWG Nichrome. The coils were assembled on the e-cig atomizer at a resistance of 1.8  \( \text{where an e-liquid formulation containing 100\% vegetable glycerin (VG) or 50:50 propylene glycol: vegetable glycerin (PG:VG) was added. Ten coils were burned at 4.3 V for 10 seconds each using a Hewlett Packard DC power supply. The aerosol was collected on a hydrophobic polytetrafluoroethylene (PTFE) membrane. This membrane was subject to Aqua Regia acid digestion and analyzed using Inductively Coupled Plasmas-Mass Spectrometry (ICP-MS).}

Aluminum-27, Silicon-28, Chromium-52, Chromium-53, Manganese-55, Iron-57, Nickel-58, and Nickel-60 were found predominantly in the aerosol that would be inhaled by the user as opposed to condensing in the atomizer. E-cigs may be supplying the user with a dose of toxic heavy metals they would normally not be exposed to when compared to traditional smoking.

This research was supported by the National Institute of Justice, Award No. 2014-R2-CX-K010 and National Institute of Health (NIH), Award P30DA033934

Keywords: Elemental Mass Spec, Forensic Chemistry, ICP-MS, Metals
Application Code: Homeland Security/Forensics
Methodology Code: Atomic Spectroscopy/Elemental Analysis
Methylmercury (CH\(_3\)Hg) is the most toxic form of various mercury species. It is produced in contaminated soils by sulfate-reducing bacteria. Natural organic matter favors the methylation process by acting as an electron donor for sulfate-reducing bacteria. Determination of trace amounts of methylmercury in heavily inorganic mercury, Hg(II), contaminated soil and sediments requires removal of Hg(II) matrix prior to measurement. In this study, an analytical approach was developed for selective determination of CH\(_3\)Hg from contaminated soils and sediments with elevated levels of Hg(II). Nitric acid was preferable over hydrochloric acid for quantitative extraction of CH\(_3\)Hg with minimal levels of Hg(II). Agitation via ultrasounds provided rapid extraction of CH\(_3\)Hg in minutes from soils as opposed to prolonged mechanical shaking. Of the total Hg(II), only about 5% was extracted from soil matrix with 5% nitric acid under same conditions. Coprecipitation was performed to eliminate the residual Hg(II) from soil extracts via hydroxide and sulfide precipitation. Among various coprecipitation schemes, ammonium sulfide was most conducive to precipitate Hg(II) effectively without impacting CH\(_3\)Hg levels. Measurements were made with cold vapor generation (CVG) inductively coupled plasma mass spectrometry (ICP-MS). Potential interferences were studied from ammonium sulfide on CVG of CH\(_3\)Hg in 5% (v/v) nitric acid. Calibration curves were established with aqueous CH\(_3\)Hg standards matched with ammonium sulfide. Linearity was achieved between 0 to 25 µg/L CH\(_3\)Hg levels. The procedure was validated by analyses of a methylmercury sediment reference material (SQC1238) and an estuarine sediment (ERM – CC580) that contained trace levels of CH\(_3\)Hg in Hg(II) matrix.

**Keywords:** Elemental Mass Spec, Environmental/Soils, Mercury, Method Development

**Application Code:** Environmental

**Methodology Code:** Atomic Spectroscopy/Elemental Analysis
The analysis of As in pharmaceutical formulations is now a requirement under the Q3D regulations. These regulations also discuss speciation analysis although little work has been carried out in this area. Arsenic speciation analysis was performed using HPLC-ICPMS equipped with a reverse phase C18 (Phenomenex 5u 250x4.60mm) column and a mobile phase consisting of 10mM tetrabutyl ammonium phosphate (TBA), 20Mm potassium phosphate monobasic (KH2PO4) and 2% methanol at pH=6. Four arsenic compounds; arsenite (As (III)), arsenate (As (V)), monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) were successfully separated using the described method. The arsenic species were detected at m/z 75 with a Thermo X-series ICP-MS.

The method was validated for linearity, accuracy and precision. The limits of detection and limits of quantification for the four compounds were: As (III): 0.095ng/ml, 0.278ng/ml, As (V): 0.119ng/ml, 0.362ng/ml, MMA: 0.122, 0.353 ng/ml and DMA: 0.118, 0.336ng/ml respectively. A selection of antacids were analysed and some products showed a total concentration of arsenic of about 800 ng/g. The arsenic was then extracted using CEM SP-D microwave with a multistep heating program where the maximum temperature was 95°C. Water and 0.3M phosphoric acid (H3PO4) were used as extraction media and H3PO4 was found to be more efficient in extracting the arsenic compounds than water. The percentage extracted for all products was more than 90% in comparison with the total concentration obtained from the reverse aqua regia extraction of the samples. As (III) was the dominant species in 5 of the 6 analysed products.

Keywords: ICP, ICP-MS, Microwave, Pharmaceutical
Application Code: Pharmaceutical
Methodology Code: Atomic Spectroscopy/Elemental Analysis
The analysis of pharmaceutical formulations for Class 1 and 2A impurities (As, Cd, Hg, Pb, Co, Ni and V) will be discussed with reference to the development of both ICP-OES and MS methods. The methods were used to analyse cold/flu remedies and analgesic tablets. Preparation of the samples using grinding and the benefits for coated tablets will be indicated. Microwave sample preparation using reverse aqua regia and the use of solid form reference material (NIST 3280) will be compared with spiked addition validation. The optimum digestion method used a three step digestion to 210°C with a CEM-SPD80.

The LoD and LoQ for each technique are provided below and were gained on a Thermo iCAP 6500 Duo for ICP-OES and an a Thermo X-Series for ICPMS

<table>
<thead>
<tr>
<th>Element</th>
<th>ICP-OES LOD</th>
<th>ICP-OES LOQ</th>
<th>ICP-MS-Std Mode LOD</th>
<th>ICP-MS-Std Mode LOQ</th>
<th>ICP-MS-CCT Mode LOD</th>
<th>ICP-MS-CCT Mode LOQ</th>
</tr>
</thead>
<tbody>
<tr>
<td>As</td>
<td>1.95</td>
<td>5.86</td>
<td>0.73</td>
<td>2.1</td>
<td>0.05</td>
<td>0.15</td>
</tr>
<tr>
<td>Cd</td>
<td>0.29</td>
<td>0.87</td>
<td>0.04</td>
<td>0.09</td>
<td>0.01</td>
<td>0.03</td>
</tr>
<tr>
<td>Hg</td>
<td>0.80</td>
<td>2.30</td>
<td>0.59</td>
<td>1.83</td>
<td>0.57</td>
<td>1.70</td>
</tr>
<tr>
<td>Pb</td>
<td>1.56</td>
<td>4.73</td>
<td>0.07</td>
<td>0.20</td>
<td>0.02</td>
<td>0.05</td>
</tr>
<tr>
<td>Co</td>
<td>0.77</td>
<td>1.58</td>
<td>0.08</td>
<td>0.22</td>
<td>0.09</td>
<td>0.29</td>
</tr>
<tr>
<td>Ni</td>
<td>0.74</td>
<td>1.72</td>
<td>0.20</td>
<td>0.59</td>
<td>0.07</td>
<td>0.23</td>
</tr>
<tr>
<td>V</td>
<td>2.75</td>
<td>7.64</td>
<td>6.33</td>
<td>19.06</td>
<td>0.15</td>
<td>0.48</td>
</tr>
</tbody>
</table>

The recoveries for both the SRM and the spiked samples were between 95-105% with RSDs of less than 5%.

The development of a solid dosage form with known metal content will be discussed as part of Coalition for Rational Implementation of the USP Elemental Impurities Requirements Technical & Analytical Challenges sub-Committee.
GC-MS analysis of volatile organic compounds (VOCs) from headspace over cell cultures has been described as useful tool for the distinction of different cell lines. However, physiological conditions such as cellular density, growth phase can influence VOC profiles. Assessing these parameters still requires expensive and often invasive and destructive biochemical methods.

To determine the effect of cell status on VOC profiles, growth of a fast proliferating murine cell line was monitored over 72 h. 20 ml headspace were pre-concentrated every 24h with polymer needle trap devices (NTDs) in triplicate. Pure medium samples were analyzed in parallel as a negative control. NTDs were analyzed with an Agilent 7890A gas chromatograph coupled to an Agilent 5975C inert XL MSD with triple axis detector. To reduce effects of biological variance between repetitions data were normalized.

The variance of the analytical method could be reduced below 10% by using a hermetically closed, inert sampling box and polymer NTDs. 14 VOCs showed differences between negative controls and cell cultures. Two compounds could be correlated to cell growth. Most VOCs were consumed during growth. VOC patterns of cell cultures depended on cellular growth and emissions from media.

Substances emitted from cell cultures mirror cell proliferation and metabolic status. Determination of VOC profiles from headspace over cell cultures may provide immediate, destruction free information on cell status.

Keywords: Bioanalytical, Biological Samples, GC-MS, Volatile Organic Compounds
Application Code: Bioanalytical
Methodology Code: Gas Chromatography/Mass Spectrometry
Extracts from the leaves of Sapium ellipticum (Se) and the stem bark of Erythrophleum suaveolens (Es) are used in the management of cancer in Alternative Medicine. The aim of this study is to analyze the essential oil extracted from each plant part by GC-MS and identify constituents which have been reported to exhibit bioactivity against cancer or are cancer-preventive. Extraction was carried out by hydrodistillation using Clevenger apparatus and the essential oil was collected into hexane. Analyses were carried out on QP-2010 Ultra Shimadzu GC-MS fitted with capillary column coated with 5% methylsiloxane using a temperature program 60 deg.C (2min.) increased to 290 at 9deg./min. The major constituent in both samples was n-hexadecanoic acid, 42% in Se and 37% in Es. Other constituents present in both samples include 9,12-octadecadienoic acid 7% in Se and 6% in Es, octadecanoic acid 4% in Se and 2% in Es and tetradecanoic acid, 3% in Se and 5% in Es. Other constituents in Se include 9,12,15-octadecatrienoic acid, 13%, and its methyl ester, 1%, phytol, 9% and squalene, 2%. Other constituents in Es include oleic acid, 5%, 1-naphthalenepropanol, .alpha.-ethyldecahydro.-alpha. 5,5,8a-tetramethyl-2-methylene, [1S-, 12%, dodecanoic acid, 4% and nerolidyl acetate, 4%. Phytol, squalene and 9,12,15-octadecatrienoic acid in Se, oleic acid and nerolidol obtainable from the acetate in Es have been reported to exhibit bioactivity against cancer. Hexadecanoic acid is an antioxidant which is cancer-preventive. These plants can be used as components for drugs for cancer management.
Deamidation is a major non–enzymatic degradation pathway for therapeutic antibodies in which the amide group of asparagine or glutamine is replaced by a carboxylic acid to form aspartic acid or glutamic acid, respectively. Deamidation may decrease protein activity and shelf life, and is therefore an important quality attribute in the biopharmaceutical industry. In our laboratory, charge variant analysis by imaged capillary isoelectric focusing (iCIEF) is conducted on an iCE 3.0 instrument (Protein Simple) for routine monitoring of deamidation.

We experienced a situation in which test IgG samples displayed an unexpected shift to increased acidic variants. Further, the test sample electropherograms appeared unusually heterogeneous, whereas the acidic species for the reference sample appear well defined. In order to determine whether these observations were associated with deamidation, the reference material and test sample were subjected to peptide mapping. Peptide mapping of a monoclonal antibody (mAb) using an LC-MS/MS approach involved the enzymatic cleavage of a purified mAb into peptides, separation by LC, and detection by tandem mass spectrometry. In our case, sample preparation involved multiple steps including denaturation, reduction, alkylation, and tryptic digestion. LC-MS/MS analysis was performed using a Waters nanoAcquity coupled to a Thermo Q Exactive Plus Orbitrap. Here we present successful correlation of the unusual charge variant profile with deamidation using peptide mapping. Additionally, the peptide mapping results provided information regarding which protein sites were deamidated.

Keywords: Bioanalytical, Capillary Electrophoresis, Characterization, Liquid Chromatography/Mass Spectroscopy
Application Code: Bioanalytical
Methodology Code: Liquid Chromatography/Mass Spectrometry
The secretion dynamics of peptide hormones is an important area of study in diabetes research. Hormones such as insulin and glucagon are released by the pancreas to maintain glucose homeostasis. Impairment in the dynamic release profile of these peptides can lead to defective glucose handling, a hallmark of the diabetic state. Currently, immunoassay methods have been used as a highly sensitive and selective method for monitoring individual peptide secretion dynamics, but this technique is limited to monitoring one or two peptides simultaneously.

This study reports on the development of a liquid chromatography mass spectrometry method to measure multiple glucose-regulating hormones simultaneously. The method utilizes a microfluidic chamber to hold islets and a pump to deliver secretagogues. The perfusate was directed to a valve where it was either collected into a sample loop or analyzed by a reversed phase C18 column using an isocratic separation followed by detection with a Micromass Quattro LC triple quadrupole mass spectrometer. The separation conditions consisted of a constant column temperature at 30 °C, a flow rate of 0.09 mL/min, and a mobile phase composition of 1:4 acetonitrile with 0.1% formic acid: water with 0.1% formic acid. The entire system was automated using an Arduino Mega 2560 microcontroller board wired to a relay board. In this report, we will present on the automated switching of the valve to accomplish continuous monitoring.

Keywords: Bioanalytical, Lab-on-a-Chip/Microfluidics, Liquid Chromatography/Mass Spectroscopy, Peptides
Bioanalytical - LC/MS
Quantification of Thymosin \( \beta_4 \) (T\( \beta_4 \)) in Rat Plasma by UHPLC-MS/MS

Introduction
Human thymosin \( \beta_4 \) (T\( \beta_4 \)), an important actin chelate molecule in eukaryotic cells, widely distributed in human tissues and cells. It is a small peptide consisted of 43 amino acid residues. Recently, the clinical trials on the traumatic diseases and cardiovascular system effects of T\( \beta_4 \) have been studied.

Methods
A sensitive and selective method for quantification of thymosin \( \beta_4 \) in rat plasma by ultra-high performance liquid chromatograph-tandem mass spectrometry (UHPLC-MS/MS) was developed. The procedure employed a simple protein precipitation step using methanol and centrifugation step. Then the supernatant sample separation was carried out using the HPLC-MS/MS 8060 (Shimadzu Corp., Kyoto, Japan). An ACQUITY UPLC® Peptide CSHTM C18 (2.1 mm I.D. × 100 mm L, 1.7 \( \mu \)m) column was used for separation. The mobile phase consisted of 0.05% formic acid (A) and 0.05% formic acid in acetonitrile (B) with a gradient elution.

Results & Discussion
This method was successfully applied to comprehensive quantification study of T\( \beta_4 \) in plasma. Calibration curves offered satisfactory linearity (\( r > 0.997 \)) within the determined ranges of 0.1-100 ng/mL. The precision was investigated by analyzing the standard and plasma samples 7 times. The results of precision based on the relative standard deviation (RSD \%) of integrated peak area, and the RSD values were both less than 8.82%. The ratio of residual area and limit of detection was 5.4\%, which meet the requirements of the guidelines for the non-clinical pharmacokinetics studies. In the present study, we have developed a rapid, highly selective, and sensitive UPLC-MS/MS method for the quantification of T\( \beta_4 \) in plasma, and the present results are useful toward the continued evaluation of T\( \beta_4 \) as a therapeutic agent.

Keywords: Bioanalytical, Drugs, Liquid Chromatography/Mass Spectroscopy
Application Code: Bioanalytical
Methodology Code: Liquid Chromatography/Mass Spectrometry
## Abstract Text

High performance liquid chromatography coupled online with mass spectrometry (HPLC-MS) is a powerful technique for analyzing a wide range of compounds and samples. The use of higher pressures in HPLC, termed ultrahigh pressure LC (UHPLC), can be beneficial for increasing both the speed and resolution of a chromatographic separation, two important parameters that can be tailored to specific samples and experiments. Capillary columns are beneficial for higher pressure separations as they limit band broadening due to frictional heating, allowing for longer columns to be employed, as well as provide nanoflow compatibility for nano electrospray ionization. In this work, a home-built UHPLC system that operates at inlet pressures up to 40,000 psi was coupled online to a high resolution mass spectrometer for metabolomic and lipidomic studies. Using capillary columns 50 – 100 cm long and packed with sub-2 [micro]m C18 particles, over 100,000 theoretical plates are attained using isocratic elution, and peak capacities of 195 in 35 minutes for pooled human serum samples using gradient elution, operating at the van Deemter optimal velocity. At higher inlet pressures, analysis time is greatly reduced while separation performance is only slightly diminished. Alternative column chemistries, including bare silica and doped reversed-phase (C18+), are also being investigated for use with metabolomic and lipidomic samples, for both targeted and untargeted analyses. The benefits of higher pressure and therefore higher efficiency separations are seen both for difficult to resolve mixtures (e.g. isomers) as well as complex samples (e.g. pooled human serum samples).

### Keywords:
- Bioanalytical, Capillary LC, Liquid Chromatography/Mass Spectroscopy, Metabolomics, Metabonomic
- Bioanalytical
- Liquid Chromatography/Mass Spectrometry
Bioanalytical - LC/MS

Development and Validation of an LC-MS/MS Nucleomics Method for Determination of Nucleotide Profiling in Escherichia Coli Lysate

Due to the different absorptivity coefficients for 2’3’ and 5’ nucleotide isomers, a simple UV absorbance method could not be used for accurate quantitation without physical separation of these isomers. A hydrophilic interaction liquid chromatography (HILIC) based nucleomics method by liquid chromatography-tandem mass spectrometry (LC-MS/MS) has been developed to quantitate these nucleotide isomers along with diphosphates, triphosphates, nucleosides and nucleobases from an Escherichia coli cell lysate matrix. Analyses were performed on an Agilent HPLC 1260 system using a SeQuant 50mmx 2.1mm x 3µm ZIC-HILIC column eluted with a 20mM ammonium acetate pH 6.8 acetonitrile-water gradient and detected using negative mode electrospray ionization with a run time of 23 minutes. Precision for intra and inter-day was less than 6% and on column limits of detection between 40 and 400ng/mL. Linear ranges spanned at least two orders of magnitude and spiked recoveries were greater than 96%. No carryover was observed for concentrations within the linear range. Using this method, kinetic studies of depolymerization and stability of polymeric RNA was studied and the data was utilized to optimize the reaction conditions.

Keywords: Liquid Chromatography/Mass Spectroscopy
Application Code: Bioanalytical
Methodology Code: Liquid Chromatography/Mass Spectrometry
Bioanalytical - LC/MS

Highly Sensitive and Selective Measurement of Underivatized Methylmalonic Acid in Serum by LC-MS/MS

Introduction

Methylmalonic acid (MMA) is a metabolic intermediate in the mitochondrial conversion of propionic acid to succinic acid (SA). Vitamin B12 (VB12) is an essential cofactor for the methylmalonyl mutase-dependent carbon rearrangement of MMA to SA. Therefore, VB12 deficiency leads to high circulating levels of MMA. VB12 deficiency is an important and often under-recognized problem, which results in irreversible neurological damage, anemia, osteoporosis, and cerebrovascular and cardiovascular diseases.

Determining serum MMA instead of VB12 present several advantages. First, despite evidence of functional VB12 deficiency, serum VB12 concentrations can be normal. Second, VB12 is less stable, which is sensitive to light, while MMA is extremely stable. Furthermore, serum MMA concentrations are much higher than VB12 levels (nM vs. pM), and thus elevated concentrations of MMA are easier to detect than decreased levels of VB12.

Method

The aim of this study was to develop a rapid, sensitive and specific method for serum MMA measurement by ultra-performance liquid chromatograph-tandem mass spectrometry (LC-MS/MS). Sample pretreatment was achieved by liquid-liquid extraction using acidified MTBE without any derivatization. Sample separation was performed on a PC HILIC (2.0 mm I.D. x 150 mm L, 5 μm) by gradient elution with a flow rate of 0.5 mL/min at 55°C. Samples were analyzed by LCMS-8050 under negative electrospray ionization mode and quantified by multiple reaction monitoring (MRM) method.

Results & Discussion

In this study, a good linearity was obtained with the relative coefficient greater than 0.997. RSDs of within-day and between-day were lower than 7.5% and 7.0%, and the accuracy ranging from 87.4 to 112.3%. It is suggested this method would be applied to clinical laboratory for serum MMA evaluation.

Keywords: Bioanalytical, Liquid Chromatography/Mass Spectroscopy, Tandem Mass Spec

Application Code: Bioanalytical

Methodology Code: Liquid Chromatography/Mass Spectrometry
Nyssa sylvatica is a tree native to the southeastern United States whose bark has been widely used by Native Americans to treat dysentery and parasitic infections. The purpose of this study is to determine the phytochemical composition of the stem and trunk bark of N. sylvatica since little is known about its phytochemistry.

Dried bark samples were chopped (<0.5 mm) or powdered and extracted with water, methanol or other solvents for phytochemical analysis. The total phenolic content (TPC) of a methanolic extract of stem and trunk whole bark was determined by the Folin-Ciocalteu method to be 39.5 mg GAE (gallic acid equivalents)/g dried bark. The total flavonoid content (TFC) using a colorimetric method was determined to be 3.5 mg RE (rutin equivalents)/g dried bark.

High performance liquid chromatography (HPLC: Shimadzu 20AD) utilizing a C18 analytical column of a methanolic (70%) extract of various stem and trunk bark layers revealed a complex variety (~53) of the hydroxycinnamate class of phenolics mainly chlorogenic acid derivatives concentrated in the 3rd bark layer adjacent to the xylem. Total hydroxycinnamate content (THC) present in this layer utilizing chlorogenic acid as standard was as follows: 65.2, 41.1, and 35.9 mg/g in small stem, large stem, and trunk respectively which correlates well with the TPC measurement. These are especially high THC levels comparable to roasted coffee beans (34-41 mg/g). An additional group of highly lipophilic HCs were found only in the 2nd bark layer of the stem and trunk. Several ellagic acid derivatives were also identified in various bark layers.

Abstract Text

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Keywords: Chromatography, Extraction, Natural Products, Spectroscopy
Application Code: Biomedical
Methodology Code: Liquid Chromatography/Mass Spectrometry
Amino acids are essential building blocks in all living organisms. Initially it was believed that only L-amino acids were relevant in higher organisms, and D-amino acids were laboratory artifacts. Today, various D-amino acids have been detected in different organisms, e.g. bacteria, plants and animals. The biological functions of some D-amino acids have been studied, and variations of D-amino acid levels have been reported in patients with different diseases, such as Alzheimer's disease and schizophrenia. D-amino acids and their regulated enzymes are becoming promising targets for drug development and potential biomarkers, especially for neurodegenerative diseases and kidney diseases. Furthermore, several studies have indicated the effect/importance of amino acids on cancer cells growth. However, there are not many fundamental studies of the enantiocomposition of free amino acids in cancer cells. Here, free L- and D-amino acid levels are determined in human breast cancer cells (MCF-7) and normal breast cells (MCF-10A). In addition, L- and D-amino acid levels in the cell culture before and after cells growth were analyzed. Moreover, the effect of different cell culture (varying amounts of glucose) on amino acid levels in cancer cells was investigated. High performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) is used for the analysis of amino acids in biological sample.
Superficially porous particle (SPP) technology has been rapidly adopted by chromatographers in the past several years due to its convincing performance advantage over totally porous particles (TPPs) such as High efficiency and lower back pressure. Whilst chromatographers enjoy the high efficiency of these superficially porous particles, there is still a significant challenge in analyzing small molecules and complex protein digests in formic acid in LC/MS with high efficiency and resolution. Formic acid is an MS-friendly ion-pairing agent that is often used in LC/MS methods, permitting sensitive detection. However, formic acid also produces broader peak shapes, which leads to the co-elution of peptides. In addition, poor peak capacity, peak broadening, and poor resolution of closely related peptides are limitations with the current formic acid containing mobile phase LC/MS methods. Hence the correct choice of reverse-phase column and LC/MS methods is critical in achieving a successful separation for small molecules and peptide mapping.

We have developed an innovative technology to hybridize superficially porous particles that are designed to resist degradation in high pH mobile phases. Based on this technology, in this presentation, we will show the synthesis of a novel type of charged organic/inorganic hybrid superficially porous particles and their benefits in LC/UV and LC/MS applications. The charged hybrid superficially porous particles bonded with C18 offered not only different selectivity and excellent chemical stability, but also excellent peak shapes and sample loading capacity, and high sensitivity and resolution when compared with conventional C18 phase, especially in formic acid condition, ideally for LC/MS application.

Keywords: HPLC, HPLC Columns, Liquid Chromatography/Mass Spectroscopy
Application Code: Bioanalytical
Methodology Code: Liquid Chromatography/Mass Spectrometry
D-amino acids, once considered as “laboratory curiosities”, have been found to be indigenous in higher organisms. The biological activity of only a few D-amino acids have been examined. The levels of certain D-amino acids in patients with different diseases, such as schizophrenia and Alzheimer’s disease, have been reported. Some D-amino acids have shown utility as potential biomarkers for neurodegenerative diseases and kidney diseases. These studies only focus on a few D-amino acids and not the spectrum of D-amino acids that are present in biological samples. As evidence grows on the biological roles of free D-amino acids, it is evident that there are limited fundamental studies on baseline levels of all free D-amino acids in biological samples. With the advent of modern analytical techniques, it is now possible to simultaneously, reliably, and quickly analyze a spectrum of free proteinogenic L- and D-amino acids. Baseline levels of all free D- and L-amino acids have been examined from a variety of blood samples. Trends and correlations of D-amino acid levels are discussed.

Keywords: Amino Acids, Bioanalytical, Chiral Separations, Liquid Chromatography/Mass Spectroscopy
Application Code: Bioanalytical
Methodology Code: Liquid Chromatography/Mass Spectrometry
Neuropeptides are involved in cell signaling and have roles in processes such as learning, memory, and neuroprotection. They are difficult to study quantitatively due to low concentrations (pM-nM) in the extracellular space and rapid degradation. Online microdialysis with cLC-MS/MS is well-suited for this work due to minimal sample handling, low detection limits, and the ability to unambiguously identify peptides. The downside is that reliable quantitation requires an isotopic standard to account for changes in ionization efficiency during analysis. Additionally, isotopic standards can be costly and challenging to introduce with online in vivo collection and detection. We have adapted an on-column dimethylation method originally developed for multiplexing proteomics samples (Raijmakers, et al., 2008) to the time scale, sample matrix, and separation conditions required for online microdialysis. Using cyanoborohydride and H2- or D2-formaldehyde, peptides are reduced and dimethylated at the N-termini and can be sequentially labeled as “sample” (light) or “standard” (heavy). This results in a m/z difference of 4 between peptides in the microdialysate and their isotopic standards, which are eluted and detected simultaneously. Additionally, we have included a D-amino acid in the perfusate for online determination of microdialysis extraction fraction and account for changes in chromatographic conditions over the course of sampling. We have demonstrated the utility of this method by quantitatively comparing the presence of leucine-enkephalin under endogenous and potassium-stimulated conditions in different spatial regions of the rat hippocampus. This technique can be easily generalized for virtually any peptide in the extracellular space, allowing reliable quantitative study of the role of neuropeptides in the brain.
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<td>Therapeutic Drug Monitoring of Tranexamic Acid in Plasma and Urine Via High Throughput Solid Phase Microextraction</td>
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<td>Primary Author</td>
<td>Nikita Looby</td>
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<td>University of Waterloo</td>
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<td>Co-Author(s)</td>
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**Abstract Text**

In this work, an SPME method was developed for the comprehensive therapeutic drug monitoring of tranexamic acid (TXA) in plasma and urine. This method – which was based on a hydrophilic-lipophillic balance (HLB) type coating – was employed for the high throughput analysis of clinical samples from patients treated with TXA undergoing cardiac surgery with the use of cardiopulmonary bypass (CPB). The aim of this investigation was to utilize the data acquired to properly correct for the currently existing dosing schedules of TXA which result in large variability of therapeutic levels among patients. This can be detrimental for those patients who experience exceedingly high levels of TXA as they can suffer from post-operative seizures. Since there are no recently reported techniques for the analysis of TXA particularly from urine, this presents a novel alternative strategy from the sample preparation aspect. The use of a more wettable coating, improved extraction time from 120 minutes (2 hours) to 5 minutes when compared to a previously developed SPME method and the use of the concept-96 for high throughput sample preparation reduced total processing time of 96 samples simultaneously from 2.8 minutes per sample to 16 s with comparable accuracy and precision.

**Keywords:** Bioanalytical, Liquid Chromatography/Mass Spectroscopy, SPME, Tandem Mass Spec

**Application Code:** Bioanalytical

**Methodology Code:** Liquid Chromatography/Mass Spectrometry
MicroRNAs (miRNAs) play a vital role in regulating gene expression and are associated with a variety of cancers, including breast cancer. Their distorted and unique expression is a potential marker in clinical diagnoses and prognoses. Thus, accurate determination of miRNA expression levels is a prerequisite for their applications. However, the assays currently available for miRNA detection typically require pre-enrichment, amplification and labeling steps, and most of the assays are only semi-quantitative. Therefore, we developed a quasi-direct liquid chromatography-tandem mass spectrometry (LC-MS/MS)-based targeted proteomics approach to quantify target miRNA by innovatively converting the miRNA signal into the mass response of a reporter peptide via a covalently immobilized DNA-peptide probe. Specifically, the probe containing the targeted proteomics-selected substrate/reporter peptide, GDRAVQLGVDPFR/AVQLGVDPFR, and the DNA sequence complementary to the target miRNA (i.e., miR-21) was first immobilized on APMTS modified silica nanoparticles using PDITC. After the immobilized probe was recognized and hybridized with the target miRNA, the excess probe was degraded using MBN and followed by a trypsin digestion of the hybrids. The reporter peptide was released and quantified using LC-MS/MS. The obtained LOQ was 5 pM. Finally, the developed assay was used for the quantitative analysis of miR-21 in breast cells and tissue samples.
Developability Assessment to Select Lead Biopharmaceuticals Using a Novel UHPLC Dual LC System

During the screening of therapeutic proteins and next generation biologics (e.g. mAbs, bispecific mAbs, fusion proteins) in search for a clinical lead molecule, it is desirable to assess their stability, physico-chemical properties and more importantly its pharmacokinetics, in order to avoid liabilities during further development. Generally, biochemical stability and long in-vivo half-live and thus long durability are desired.

To assess these properties state of the art characterization of biotherapeutics and the application of multiple and diverse chromatography techniques are required. This is both time consuming and a risk for sample stability and measurement reproducibility when the separations are run subsequently in one instrument. Here we use Thermo Scientific™ Vanquish™ UHPLC Dual LC technology addresses these conflicts by combining two individual flow paths in the footprint of a single system. Each flow path consists of a pump, an injection unit, a column and a detector. These two flow paths can be operated independently so that this Dual LC technology doubles the number of assays in the same time period.

Using this state-of-the-art, high-throughput UPLC-system we used a novel analytical column to establish a novel predictive assay to identify next generation biologics with fast clearance during the lead selection and protein engineering phase. This novel assay is combined with in-silico and further high-throughput small-scale in-vitro methods to perform a “Developability Assessment” for the selection of a new lead molecule, bridging Research and Development.

Keywords: Bioanalytical, Biopharmaceutical, HPLC, HPLC Columns

Application Code: Bioanalytical
Methodology Code: Liquid Chromatography
Separation and Characterization of Recombinant Human IgG1 NIST Monoclonal Antibody Structures and Variants Using a Suite of Chromatographic Methods

Abstract Text

For the biopharmaceutical industry, characterization of protein structure is critical to the development of safe and effective therapeutics. Variations in protein structure may arise during fermentation, post-manufacturing purification and processing, storage, and shipping. Monoclonal antibodies (mAb’s) are a specific example and one of the fastest growing protein therapeutics in today’s market. Structural variants of the final mAb product can have adverse effects on the efficacy of the mAb as well as potentially adverse effects for patients. For these reasons, it is important to understand the types and quantities of structural variants that exist. To separate and characterize variants, chromatographic methods and columns are commonly employed during clone selection during development and qualification of product prior to delivery for therapeutic use.

Here we demonstrate the separation of a model mAb and its variants using six common HPLC-based chromatography columns: Protein A capture and release, size exclusion (SEC), hydrophobic interaction (HIC), cation exchange (CEX), and reversed phase (RP) chromatography. The NIST (National Institute of Standards and Technology) mAb was selected for analysis as it is a standardized mAb with set specifications and it is also commercially available. First, we demonstrate the use of a protein A column to generate a sample concentration curve that can be used to determine the mAb concentration of a specific sample. SEC is used to separate and quantify mAb monomer, aggregates, and fragments. HIC separates protein variants based on their native surface hydrophobicity. For CEX, we demonstrate the separation of variants using a salt gradient and a pH gradient using a weak cation exchange column. Finally we show the reversed phase separation of the mAb, which separates variants based on the hydrophobicity of the denatured protein. The merits of each separation method for the analysis of the mAb will be discussed.
FDA defines biosimilar as a “biological product that is approved based on a showing that it is highly similar to an FDA-approved biological product, known as a reference product, and has no clinically meaningful differences in terms of safety and effectiveness from the reference product” (Ref. https://www.fda.gov/drugs). Size exclusion chromatography (SEC) is primarily used for the monomer of the biotherapeutic from its dimer and higher order aggregates as well as the fragments, be it an innovator drug or its biosimilar. SEC is routinely used for quality control analysis, as it is used for corresponding innovator drugs. Here we report the use of a 2 µm 4.6 mm ID × 30 cm Silica based SEC column for the analysis of a number of biosimilar and the corresponding innovator drugs, humira, Herceptin, avastin and Yervoy. System suitability, reproducibility in typical peak parameter such retention time (RT) peak area etc., were established during method development. An excellent linearity over the experimental range could be obtained. Limit of detection (LOD) and Limit of Quantitation (LOQ) were also compared. Overall a robust and simple method for the characterization of biosimilars is reported.

Abstract Text

Bioanalytical - Liquid Chromatography

Analytical Characterization of Biosimilars Using a 2 µm Silica Based Size Exclusion Chromatography Column

Primary Author
Atis Chakrabarti
Tosoh Bioscience LLC

Keywords: Bioanalytical, HPLC Columns, Liquid Chromatography, Protein
Application Code: Bioanalytical
Methodology Code: Liquid Chromatography
In the current scientific environment, there is a need for rapid and robust high throughput analysis of biotherapeutics, in particular monoclonal antibodies (mAbs). Although standard UHPLC systems can analyse samples simply and rapidly, there is typically only the option to use one stationary phase at any given time, resulting in prolonged sample analysis time and additional costs. Here, the newly developed Thermo Scientific™ Vanquish™ Dual LC technology with a dual pump, a dual sampler, two columns and two detectors was employed to demonstrate robust and reproducible, high-precision chromatographic analysis of monoclonal antibodies. The UHPLC system offers two fully separated fluidics which do not interfere with each other and combines the functionality of two systems in the foot-print of one.

Two identical size exclusion chromatography (SEC) columns were employed for the simultaneous analysis of the therapeutic monoclonal antibody bevacizumab to demonstrate the high throughput capabilities of the Dual LC technology. This approach enables double the throughput of time consuming SEC analysis for large number of samples to be monitored for aggregation. Using the same mobile phase and method parameters, 100 injections of the mAb sampled from the same vial were performed on each column. Excellent retention time precision and data confidence were achieved with outstanding RSD values and no loss of sensitivity.

To demonstrate the flexibility of the Dual LC technology, two orthogonal stationary phases (ion exchange/SEC and reversed phase/ion exchange) were employed for the analysis of bevacizumab (SCX/SEC) and infliximab (RP/SCX). Ten injections of the mAb from the same vial were analysed on each column simultaneously using the relevant mobile phases and method parameters. High precision and good data confidence were obtained for both columns. The capabilities of this dual column setup resulted in analysis time twice as fast as a standardly used UHPLC system.

Keywords: Biotechnology, Pharmaceutical, Protein
Application Code: Bioanalytical
Methodology Code: Liquid Chromatography
Bioanalytical - Liquid Chromatography

Development of High Resolution SEC Column Suited for Analysis of Monoclonal Antibody Aggregates and Fragments

The recent development of bio-pharmaceutical industry has been remarkable, and shortening the development time and reducing the cost become increasingly important. Antibody drugs are expected to enhance the effect of therapies without adverse side effects. During the manufacturing processes of immunoglobulin G (IgG), which is a main ingredient of antibody drugs, its aggregates and fragments could be generated as impurities. Size exclusion chromatography (SEC) analysis is known as a common method for determination of IgG characteristics as well as quality control in the production processes of IgG. It is generally known that larger pore volume of silica base contributes to higher resolution, and the smaller particle size contributes to higher theoretical plate number in SEC column. So the more suitable silica base was selected in accordance with above mentioned story to develop high performance SEC column. The new SEC column with the selected silica base was tested by monoclonal antibody analyses. The column showed high resolution between aggregates, main antibody and fragments. The effect of increase in pore volume of silica base was also confirmed as a gentle slope of protein calibration curve. In this poster, we will introduce more detailed features of the newly-developed SEC column, and analyses of monoclonal antibodies by using it.

Keywords: Biopharmaceutical, HPLC
Application Code: Bioanalytical
Methodology Code: Liquid Chromatography
Cancers, originating from gene mutations in a single cell, is hardly detectable until it is beyond control. Challenged by the sensitive, selective and accurate diagnosis and therapy, smart and robust molecular probes capable of discriminating cancer-related molecular signatures in living biosystems are urgently desired. With the respect to the effectiveness of chemical tools for cancer targeting, a specific peptide AP2H towards a tumor-related protein lysosomal protein transmembrane 4 beta (LAPTM4B) was rationally designed and efficiently evolved by the combination of HPAC screening, RP-HPLC separation and mass spectrometry characterization. Capable of discriminating LAPTM4B and responding to tumor acidosis, AP2H was successfully utilized as the targeting vehicle for the fabrication of multifunctional bioprobes for tumor treatments. The incorporation of an aggregation-induced emission fluorophore greatly facilitated the real-time tracing and subcellular localization of the target protein in living cells with very high signal-to-noise ratio. Dual-functional peptide probes were also tailored for image-guided photodynamic ablation of cancer cells. Taking advantage of the abundant chemistries of peptide conjugates, a peptide-based prodrug was further developed. The integration of the tumor-specific peptide, doxorubicin and a pH-sensitive bridge fulfilled targeted drug delivery and in situ activatable drug release in cancer cells. Our peptide-guided systems provide a promising platform for the design of more sensitive and accurate theranostic approaches for broad-spectrum cancer treatment.
Bioanalytical - Liquid Chromatography

HPLC Determination of Thiol Drug Penetration in Ocular Tissues for Development of Non-invasive Cataract Treatment

Cataract accounts for more than 50% of blindness worldwide, with an estimated 5 million new cases each year. There is now a high demand for an eye drop formulation to effectively treat or prevent cataracts in those for whom surgery is not a viable option, especially in countries facing economical barriers and limited access to surgeons. Cataract is thought to be caused by the aggregation of lens proteins as a result of oxidative damage. Thiol antioxidants have demonstrated the potential to prevent the formation of cataracts when administered before and during oxidative insults; however, due to various anatomical obstacles, achieving therapeutic concentrations in the lens is difficult and poses a major challenge to drug-based therapy. The objective of this study was to validate and apply sensitive chromatographic methods for the purpose of evaluating the extent of corneal penetration achieved by various thiol antioxidants and to determine their ability to accumulate in the lens and peripheral tissues. Eye drops were administered to the eyes of Wistar rats with and without selenite-induced cataracts of varied severity. Ocular tissues were collected 0.5-2 hours after administration of eye drops for analysis. ThioGlo-3 was used for pre-column derivatization of samples, and quantification was performed by fluorescence detection. Separation was conducted on either a Kinetex C18 core-shell column (4.6 mm x 75 mm) with 2.6 μm particles or an Orochem Reliasil C18 column (4.6 mm x 250 mm) with 5.0 μm particles, depending on the drug being analyzed. Tiopronin, N-acetylcysteine, and N-acetylcysteine amide accumulated in lenses in excess of 10 nmol/mg of protein when lenses were incubated in medium supplemented with the drugs in vitro, but drug accumulation was minimal in the lens and posterior tissues after administration of eye drops. These results highlight the need for effective drug delivery vehicles in order for a drug-based approach to be viable.

This work was supported by the Richard K. Vitek endowment and a generous donation by Dr. Helen Abadzi.

Keywords: Bioanalytical, HPLC, Method Development, Pharmaceutical

Application Code: Bioanalytical

Methodology Code: Liquid Chromatography
Monoclonal antibody development scientists need to screen the harvested cell cultures for selecting the best clone with high expression level by titer analysis. A variety of monoclonal antibodies from harvested cell culture media can be captured and accurately quantitated by Protein A affinity chromatography. Harvested cell cultures from upstream are full of impurities which need to be removed to get a partially purified mAb with minimum residual impurities for subsequent downstream purification steps. Chromatographers use 20 μm, 4.6 mm ID × 3.5 cm column Protein-A column for this purpose of partial purification of monoclonal antibody. Immunoglobulins exist in various types and subtypes. Interest in using these varieties of IgGs, other than IgG1 for bio therapeutic purpose is rapidly growing. Here in our study we have used a recombinant protein-A column with code-modified hexamer of the C domain which some other recombinant protein-A ligands do not possess. Rapid purification of varieties of IgG subclasses such as IgG1, IgG2, IgG4 as well as IgGs from a number of different sources such as human, mouse, rat and rabbit is discussed. Fast separation within 2 minutes, robust quantification of antibodies, and long lifetime of more than 2000 injections per column with no signs of deterioration could be carried out using this Protein-A affinity chromatography column.

Keywords: Bioanalytical, HPLC Columns, Liquid Chromatography, Protein
Application Code: Bioanalytical
Methodology Code: Liquid Chromatography
Bioanalytical - Liquid Chromatography

Using Temperature Assisted Solute Focusing to Improve Detection of Neuropeptides in Microdialysates

Neuropeptides play important roles in cell signaling but are difficult to measure due to small (pM to nM) concentrations in the extracellular space. Microdialysis is useful for collecting neuropeptides but typical recovery for peptides is low, thus concentrations in dialysate may be too low to measure without effective preconcentration. This can be achieved using capillary liquid chromatography, which uses small column diameters (<150 μm) to minimize radial dilution and increase sensitivity compared to analytical scale columns. Large injection volumes help with detection of trace analytes but can lead to volume overload, which decreases resolution and sensitivity for early peaks. Temperature-Assisted Solute Focusing (TASF) is a focusing technique developed in our lab which minimizes the effects of volume overload by using a thermoelectric cooler (TEC) to cool the column head to 0°C during injection. This traps and focuses analytes at the head of the column. After injection, the temperature of the TEC is rapidly raised to 60°C to achieve separation in a timely manner.

In this study, retention factors of ten neuropeptides, including angiotensin I, II, III, and IV and the enkephalins, were measured in four mobile phase compositions and at five temperatures. We then used van’t Hoff plots to calculate the adsorption enthalpies of each peptide. We also fit the data to a retention model by Neue and Kuss to extrapolate retention factors for TASF simulations. Our simulations show that for an injection volume 100x larger than the column volume, TASF can separate all ten neuropeptides in two minutes. These injection volumes are required to achieve a quantifiable signal with mass spectrometric detection. In analogous isothermal separations only 6 neuropeptides were resolved and the signal decreased by 45% for some analytes. Thus, our simulations suggest that TASF is a useful tool for

Abstract Text

Keywords: Bioanalytical, Capillary LC, Neurochemistry, Peptides

Application Code: Bioanalytical

Methodology Code: Liquid Chromatography
Bioanalytical - Mass Spectrometry


Mass spectrometry imaging (MSI) has recently advanced to allow the visualization of metabolites at cellular and sub-cellular resolution, unveiling unprecedented details of metabolic biology. Recently, we have achieved high-spatial resolution matrix-assisted laser desorption/ionization (MALDI)-MSI down to 5 µm by modifying the laser optics of our linear ion trap-Orbitrap mass spectrometer. This work demonstrates, for the first time, that high-resolution MALDI-MSI can be applied for three dimensional chemical imaging of a single cell using newly fertilized individual zebrafish embryos as a model system. The zebrafish (Danio rerio) has been widely used as a model vertebrate system to study lipid metabolism, the roles of lipids in diseases, and lipid dynamics in embryonic development. High-spatial resolution MALDI-MSI was used to map and visualize the three-dimensional spatial distribution of phospholipid classes, phosphatidylcholines (PC), phosphatidylethanolamines (PE), and phosphatidylinositols (PI), in the zebrafish embryo. All three phospholipid classes are present with symmetric distribution inside the blastodisc, as well as the boundary of the yolk, but each reveals different localizations; for example, PE shows heterogeneous sub-cellular localization highly abundant at the center of the blastodisc. The 3D MALDI-MSI volumetric reconstructions were then used to compare four different normalization approaches to find reliable relative quantification in 2D- and 3D- MALDI MSI data sets. Furthermore, two-dimensional MSI was studied for embryos at different cell developmental stages (1-, 2-, 4-, 8-, and 16-cell stage) to investigate the localization changes of some lipids, revealing heterogeneous localizations of different classes of lipids in the embryo.

This work is partially supported by the US Department of Energy (DOE), Office of Biological and Environmental Research.

Keywords: Imaging, Lipids, Mass Spectrometry
Application Code: Bioanalytical
Methodology Code: Mass Spectrometry
Population Health Outcomes Measured Through Wastewater Epidemiology

Wastewater is akin to a pooled urine sample, and therefore contains a wealth of information regarding the chemicals excreted by a community. Wastewater epidemiology using LC-MS/MS has been widely applied to objectively measure drug use in many communities around the globe. Wastewater epidemiology has also been proposed as a tool to measure population health outcomes. Achieving this is the aim of this project. Targeted LC-MS/MS methods were developed to quantify a panel of potential "biomarkers" related to population health and stress. Temporal and spatial trends of these biomarkers were investigated in wastewater sampled from a range of catchments and time points in Australia. Persistent geographic patterns were evident for the per capita loads of “health burden” biomarkers such as antidiabetics, antihistamines and tobacco metabolites. Small but consistent differences were seen across different catchments for biomarkers such as the metabolites of vitamins B3, B6 and K. Correlations were found between biomarkers of cause and effect, such as the histamine metabolite 1-methyl-4-imidazoleacetic acid and the antihistamine fexofenadine. Per capita loads of some biomarkers were difficult to contextualise, highlighting the need for triangulating wastewater analysis data with allied health or socioeconomic data. This project is a first key step towards developing wastewater epidemiology into a tool to measure population-scale health outcomes in an objective, non-invasive and cost effective manner.

Keywords: Beverage, Environmental/Water, Food Science, Mass Spectrometry
Application Code: Bioanalytical
Methodology Code: Mass Spectrometry
Cytochrome C Vulnerability to Malondialdehyde Modification: A Study Focusing On Mass Spectrometry

Malondialdehyde (MDA) is a highly reactive compound shown to cause toxic stress in cells and to yield covalent protein adducts, referred to as advanced lipoxidation endproducts. The objectives of this study were threefold: 1) to investigate the reactivity of MDA with cytochrome c (Cyt c); a protein found in the electron transport system, 2) to identify the amino acids in Cyt c vulnerable to non-enzymic attack by MDA, and 3) to determine the effects of MDA on the secondary structure of the protein. Studies using UV and mass spectrometry demonstrated the high reactivity of MDA, and revealed that the extent modification of Cyt c by MDA was a time dependent process; a process relying on the exposure period of Cyt c with MDA.

Mass spectrometric data further revealed that the coupling of Cyt c with MDA occurred via certain lysine residues, with K13, K27, and K88 implicated in the process. The CD spectrum of native Cyt c yielded a profile typical for helical proteins, with bands at 222 nm, 208 nm and 190 nm. A comparison of the CD spectrum of the native protein with the CD spectra of Cyt c modified with MDA at various concentrations showed no difference between all the spectral profiles; a result demonstrating that the coupling of MDA with Cyt c instigated no changes in the protein’s secondary structure. These studies warrant further investigation as MDA has been shown to form in the mitochondria, and to be implicated with aging and neurodegenerative diseases.

Keywords: Bioanalytical, Biomedical, Proteomics, Spectrophotometry
Application Code: Biomedical
Methodology Code: Mass Spectrometry
In this study, we report a method for ultrasensitive detection of chiral molecule L/D-carnitine based on chirality-transfer phenomenon. The method has been demonstrated to work at the single molecule level where the binding between self-assembled cysteine-gold nanoclusters (Cys-Au NCs) and the analytes molecule. This process was observed to L-Cys-Au NCs bind with D-carnitine and D-Cys-Au NCs bind with L-carnitine, which can be used to identify the analytes of interest. The molecular recognition mechanism between cysteine and carnitine has been studied. We have employed LDI-MS to detect Cys-Au NCs binding with carnitine cells through the monitoring of Au cluster ions ([Au\(_n\)]\(^+\); 1 \(\leq n \leq 3\)). The ultrahigh signal from Au NCs through the formation of a number of [Au\(_n\)]\(^+\) ions results in a sensing platform with M level carnitine. Further, we have applied the L-Cys-Au NCs and D-Cys-Au NCs as a labeling agent for D-carnitine and L-carnitine, respectively. The results suggested that a nanocluster-assisted LDI-MS platform was successfully built and that such a device can be used for ultrasensitive detection of chiral molecules.
As profiles of breath biomarkers in animals may significantly differ from those in humans, influences on VOC exhalation have to be characterized when translational animal models are to be established. In this study changes in VOC composition in breath of cattle were assessed over the day by means of proton-transfer-reaction time-of-flight mass spectrometry (PTR-ToF-MS).

Breath was sampled for three minutes every 2 hours over 10 hours via a tightly fitting face mask, connected to PTR-TOF 1000. Discrimination between inspiratory and expiratory breath phases was realized through a custom made data processing algorithm. Thirty-six VOCs were monitored over the period of 10h. Substance specific changes over the day were observed (e.g. (C[3]H[6]O)H+ increased by 60% and (C[4]H[8]O)H+ by 150%) Due to the sudden increase of some VOCs during the physiological eructation some substances (e.g. (C[5]H[10])H+ by 45 fold and returned to initial values within the following breaths. Decrease after eructation was substance specific (from 4% to 24% per breath).

An additional data algorithm called “Burb Tracker” was developed to discriminate eructation and normal breath phases. Day profile of VOC’s by means of real-time analysis allowed detection of metabolic changes as well as recognition of specific phases in the breath profile such as burbs. Data processing algorithms enable discrimination of VOCs coming from rumen or breath. In a perspective, the results enhance basic understanding of the origin of exhaled VOCs in animals.

Keywords: Bioanalytical, Time of Flight MS, Trace Analysis, Volatile Organic Compounds
Application Code: Bioanalytical
Methodology Code: Mass Spectrometry
In vitro cell models can be used to investigate altered central metabolic networks that contribute to cell proliferation, growth and survival in cancer. Clear cell renal cell carcinoma (ccRCC) is the most common (75%) histological subtype of kidney cancer. In this study, we have optimized a protocol for harvesting, and extraction of extracellular metabolites from human renal cell lines, and we have utilized a discovery-based metabolomics approach to profile the exometabolome by means of ultraperformance liquid chromatography coupled to quadrupole-time-of-flight mass spectrometry (UPLC-QTOF-MS). Conditioned media were obtained from a non-tumor human embryonic kidney cell line, HEK-293, which was used as control; and from a ccRCC cell line 786-O, which derives from primary ccRCC tumor. A total of 22 conditioned media samples were analyzed for each cell type, including 2 biological replicates. Metabolite extracts were lyophilized and reconstituted prior to reversed-phase UPLC-QTOF-MS analysis. Metabolic features (Rt, m/z pairs) were analyzed using a cross-validated orthogonal projection to latent structures-discriminant analysis model using a genetic algorithm variable selection method. A panel of 12 compounds with tentative chemical identification distinguished sample classes with 100% specificity, sensitivity and accuracy. In addition, 5 of these compounds were present in 10 human serum samples from ccRCC patients and healthy controls. Discriminant metabolic features suggest alterations of the glutathione, and phenylalanine metabolism, and alteration of the tryptophan degradation pathway and the pentose phosphate pathway. Our current work involves the validation of the tentative identification with chemical standards.

We acknowledge CONICET, ANPCYT, and MINCYT for providing the funding and the Public Oncologic Serum Biobank from Instituto de Oncología A. H. Roffo for providing the samples.
Bioanalytical - Mass Spectrometry

Thermal Ionization Mass Spectrometric Quantification of Radioactive Strontium-90 Using Isotope Dilution-Total Evaporation Method

In radionuclide analysis for decommissioning of Fukushima Dai-ichi Nuclear Power Station, a rapid analytical system for the [beta]-rays emitter, particularly [sup]90[/sup]Sr is required. Typical radiometric analytical method (ex; milking-scavenging-low background gas-flow counter) need long time to determine [sup]90[/sup]Sr concentration in samples. Recently, a mass spectrometric quantification method for [sup]90[/sup]Sr was reported. It can be measured in very short time; however, the analytical sensitivity of [sup]90[/sup]Sr gradually decreases with the increase of stable Sr concentration. Since [sup]90[/sup]Sr is not able to be separated from stable isotope by chemical separation, the physical approach such as mass separation is required. To overcome the problems, we have focused on the use of thermal ionization mass spectrometry (TIMS) which can measure the isotope ratios in high precision. In this study, an isotope dilution method (ID) and total evaporation (TE) method was adopted into TIMS system and the quantification of an ultra-trace amount of [sup]90[/sup]Sr in the presence of high concentration of stable Sr was conducted by this system. This system can measure 1 µL sample volume and the analytical run time was 30-40 min containing the process of ion beam tuning (10 min). As comparison with typical ID-TIMS, the repeatability (relative standard deviation (RSD)) was 0.147 and 0.109% for ID-TIMS and ID-TE-TIMS, respectively. In the result of the addition and recovery test, [sup]90[/sup]Sr was found at 4.52 mBq, when 1 µL of 3.83 mBq [sup]90[/sup]Sr was spiked). As the results, the quantification values were highly accurately corresponded with additional amount.

Session Title
Bioanalytical - Mass Spectrometry

Abstract Title
Thermal Ionization Mass Spectrometric Quantification of Radioactive Strontium-90 Using Isotope Dilution-Total Evaporation Method

Primary Author
Chihiro Ito
Fukushima University

Co-Author(s)
Katz Suzuki, Shigeyuki Wakaki, Takashi Miyazaki, Yoshitaka Takagai

Abstract Text
In radionuclide analysis for decommissioning of Fukushima Dai-ichi Nuclear Power Station, a rapid analytical system for the [beta]-rays emitter, particularly [sup]90[/sup]Sr is required. Typical radiometric analytical method (ex; milking-scavenging-low background gas-flow counter) need long time to determine [sup]90[/sup]Sr concentration in samples. Recently, a mass spectrometric quantification method for [sup]90[/sup]Sr was reported. It can be measured in very short time; however, the analytical sensitivity of [sup]90[/sup]Sr gradually decreases with the increase of stable Sr concentration. Since [sup]90[/sup]Sr is not able to be separated from stable isotope by chemical separation, the physical approach such as mass separation is required. To overcome the problems, we have focused on the use of thermal ionization mass spectrometry (TIMS) which can measure the isotope ratios in high precision. In this study, an isotope dilution method (ID) and total evaporation (TE) method was adopted into TIMS system and the quantification of an ultra-trace amount of [sup]90[/sup]Sr in the presence of high concentration of stable Sr was conducted by this system. This system can measure 1 µL sample volume and the analytical run time was 30-40 min containing the process of ion beam tuning (10 min). As comparison with typical ID-TIMS, the repeatability (relative standard deviation (RSD)) was 0.147 and 0.109% for ID-TIMS and ID-TE-TIMS, respectively. In the result of the addition and recovery test, [sup]90[/sup]Sr was found at 4.52 mBq, when 1 µL of 3.83 mBq [sup]90[/sup]Sr was spiked). As the results, the quantification values were highly accurately corresponded with additional amount.

Keywords: Environmental, Mass Spectrometry, Method Development, Nuclear Analytical Applications

Application Code: Nuclear

Methodology Code: Mass Spectrometry
Ethylbenzene and the three xylenes are isobaric compounds (C8H10) that are traditionally difficult to distinguish using direct mass spectrometric detection. To achieve separation, chromatography is usually utilized prior to mass spectrometric detection and quantitation. However, chromatography necessarily leads to long analysis times and low sample throughput, plus it requires highly skilled technical staff. This is a significant issue for industrial applications; in particular, for vehicle manufacturers who need to meet new requirements for vehicle interior air quality (VIAQ) from China. The new Chinese regulations impose different concentration thresholds for ethylbenzene and the total xylene isomers, requiring that they be differentiated even in initial screening. Direct, real-time analysis and simple operation are critical for successfully addressing these challenges. Recently, selected ion flow tube mass spectrometry (SIFT-MS) has been applied to the differentiation of ethylbenzene from the xylene isomers. SIFT-MS is a direct mass spectrometric method that utilizes known ion-molecule reactions of eight mass-selected reagent ions to detect and quantify volatile compounds and has the potential to do so.

This paper describes the investigation of recently introduced negatively charged reagent ions for selective analysis of ethylbenzene and the total xylenes, because positively charged ions do not. The study has found that the OH⁻[sup]-[/sup] reagent ion enables the ethylbenzene isomer to be distinguished from the xylene isomers because different product masses are formed (m/z 33- and 105-, respectively). The experimental results reported here were supported by calculated optimized geometries and energies using quantum mechanical calculations (employing the B3LYP D3/6-31++G(d,p) method). Rapid differentiation of these species using SIFT-MS will greatly improve the turnaround time for screening vehicle cabins.

Keywords: High Throughput Chemical Analysis, Industrial Hygiene, Trace Analysis, Volatile Organic Compounds
Application Code: High-Throughput Chemical Analysis
Methodology Code: Mass Spectrometry
**Bioanalytical - Mass Spectrometry**

**Tandem Ionization: Complementary Mass Spectra for Confident Identification of Isomers**

Electron ionisation (EI) at 70 eV is standard practice for GC-MS, due to the high degree of ionisation (and thus good sensitivity) and consistent mass spectra generated.

Nevertheless, the identification of individual compounds in complex samples remains challenging when multiple compounds in a chemical class have similar spectra, or weak molecular ions at 70 eV EI. This problem can be addressed by the use of soft ionisation to reduce the degree of ion fragmentation, but this approach has been cumbersome to implement until now.

The mass spectrometer used in this study incorporates a unique ion source design enabling both hard and soft electron ionisation spectra to be acquired simultaneously – in a technique deemed Tandem Ionisation.

The soft EI spectra consist of only structurally-significant fragments for reduced demand on dynamic range and an increase in peak capacity. In addition, greater orthogonality between the mass spectra of isomeric compounds is evident, thus simplifying compound identification and reducing reliance on retention indices.

**Keywords:** Characterization, Gas Chromatography/Mass Spectrometry, Mass Spectrometry, Time of Flight MS

**Application Code:** General Interest

**Methodology Code:** Mass Spectrometry
**Abstract Title**: Rapid Food Analysis: A New Speed Level in Proton-Transfer-Reaction Mass Spectrometry

**Primary Author**: Lukas Märk, Ionicon Analytik

**Co-Authors**: Alfons Jordan, Felix Piel, Gernot Hanel, Jens Herbig, Klaus Winkler, Paul Mutschlechner, Philipp Sulzer, Rene Gutmann, Simone Jürschik, Stefan Feil

**Abstract Text**

Here we introduce a series of new developments for the on-line detection of volatile organic compounds (VOC), which offer considerable benefit for users who have high sample throughput and/or need increased measurement speed (good S/N due to high sensitivity).

First we present a modular ion funnel, which can be installed in a conventional Proton-Transfer-Reaction Time-Of-Flight Mass Spectrometer (PTR-TOFMS) in order to boost the sensitivity by up to one order of magnitude. An ion funnel is an electronic device which consists of a series of consecutive lenses with decreasing apertures and an applied RF voltage. The ion funnel, which is installed between the reaction region and the TOF mass spectrometer, has a strong focusing effect and thus improves the efficiency of ion transfer. Importantly, the ionization conditions are not considerably affected, so that branching ratios remain comparable to PTR-MS instruments without ion funnel.

To increase the mass resolution to up to 6000 $m/z$ and the sensitivity to higher than 1000 cps/ppbv the ion funnel has been complemented by a hexapole ion guide in another newly developed instrument (PTR-TOF 6000 X2). Especially in complex matrices, e.g. found in food and flavor research, the high mass resolution enables the identification of isobaric compounds (Fig. 1).

This project was supported by the EU IMPACT (GA 674911), the Land Tirol via a FuEul project and the Austrian Research Promotion Agency (FFG) via a Basisprogramm and an ASAP project.

**Keywords**: Food Science, Instrumentation, Mass Spectrometry, Volatile Organic Compounds

**Application Code**: General Interest

**Methodology Code**: Mass Spectrometry
The physical and chemical properties of carbon nanotubes (CNTs) make it the most widely used nanomaterial suitable for its biological and industrial applications. The widespread use of products that contain CNTs can generate the potential for release of CNTs into the environment and increase human exposure. Safety assessment including potential impacts on human health and the environment along with several toxicity tests and releasing scenarios have been reported. To understand the biocompatibility, toxicity, and risk assessment of CNTs additional work related to quantification of metal impurities still needs to be accomplished. This study herein describes the use of Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) to quantify multi-element impurities in carbon-based structures such as graphene, graphite, and carbon nanotubes. Prior to ICP-MS analysis, samples were prepared by conventional microwave acid digestion (CMAD) and microwave oxygen combustion (MOC). In addition, the ICP-MS method was validated by determining multiple analytical figures of merit such as linear calibration range, system stability, method detection limit (MDL), limit of quantitation (LOQ), and accuracy. Both of the developed CMAD and MOC methods worked well to digest metal impurities in carbon nanotubes. The ICP-MS results were in good agreement with each other and with the certified values. While comparing the two digestion methods, CMAD is simple, straightforward two-step procedure with safe experimental conditions and requires only a few milligrams of sample. Moreover, CMAD overcomes the disadvantage of the pre-existing CNT digestion methods such as the requirement of multi-step acid digestion and drying processes. We anticipate that the developed methods can assist in the assessment of material safety for health risk and science-based regulation of products containing carbon nanotubes.

Keywords: Elemental Analysis, ICP-MS, Nanotechnology, Sample Preparation
Application Code: Nanotechnology
Methodology Code: Mass Spectrometry
Bioanalytical - Mass Spectrometry

Determination of Hydromorphone in Bearded Dragon Plasma using HPLC-MS Detection

A new method of analysis has been developed and validated for the determination of hydromorphone concentrations in bearded dragon plasma samples. Following solid phase extraction, samples were separated by reversed-phase high-performance liquid chromatography on an Atlantis T3 column (4.6 x 150 mm, 3 [micro]m) and detected by mass spectroscopy. The mobile phase was a mixture of water with 0.1% formic acid, and acetonitrile with 0.1% formic acid (91:9), with a flow rate of 0.7 mL/min. The procedure produced a linear curve over the concentration range of 1 to 500 ng/mL for hydromorphone in bearded dragon plasma with an limit of detection of 0.5 ng/mL and limit of quantification of 1 ng/mL. Intra-assay variability ranged from 1.2%-7.6% while inter-assay variability ranged from 2.9%-4.7%. The average recovery for hydromorphone was 91% while the average recovery for naltrexone, which was used as the internal standard, was 90%. This method has been developed in bearded dragon plasma and should be applicable to other species, making it useful to those investigators dealing with small sample volumes, particularly when conducting pharmacokinetic studies that require multiple sampling from the same animal.

Keywords: Drugs, HPLC, Mass Spectrometry, Validation

Application Code: Validation

Methodology Code: Mass Spectrometry
### Abstract Text

The pyridinium-based amino acids, desmosine (Des) and its structural isomer isodesmosine (Isodes) stemming from the condensation of four lysine amino acid residues, serve as a cross-linking network of elastin. The elastin protein contributes to the structural foundation of lung tissue, skin and blood vessels, and provides elasticity so that tissues can maintain their shape and normal physiological functions. Quantification of elastin degradation products, desmosine and isodesmosine may be used as a biomarker for various pathological conditions including chronic obstructive pulmonary disease (COPD). The current study presents a novel approach to quantify desmosine and isodesmosine using Matrix-Assisted Laser Desorption Ionization (MALDI)-tandem mass spectrometry (MS2). MALDI-MS2 analysis of desmosine and isodesmosine are performed using stable-isotope labeled desmosine as an internal standard in different biological fluids such as urine and calf serum. Selectivity is obtained by directly investigating the m/z range associated with unlabeled desmosine and its isotope labeled counterpart. The time for 10 samples in terms of preparation and instrumental analysis took one hour which approximately equates to the associated time for 1-2 comparable liquid chromatography –mass spectrometry runs. We demonstrate linearity over more than an order of magnitude of concentration, run-to-run reproducibility over a number of weeks and different investigators, and limit of detection into the mid-femtomole range without prior concentration of sample. Finally we show the effectiveness of the method for detecting desmosine in water, plasma and urine. Des degradation upon UV radiation (254nm) at different time points is performed using the MALDI-MS2 method and NMR.

### Keywords:
- Bioanalytical
- Ion Trap
- Mass Spectrometry
- Tandem Mass Spec

### Application Code:
- Bioanalytical

### Methodology Code:
- Mass Spectrometry
Aliphatic amines represent an important class of compounds that can be found in biological fluids. Amines in exhaled breath can be related to disease, metabolic processes and bacterial activity. The high reactivity of aliphatic amines induce problems in sample preparation, analysis and quantification. Real-time analysis by means of direct mass spectrometric techniques represent an innovative approach to avoid problems related to sample preparation and storage.

We developed a method for real-time breath monitoring of methylamine, dimethylamine and trimethylamine using a PTR-ToF-MS 8000. Gaseous standard solutions were prepared in Tedlar bags through dilution with pure nitrogen and using a liquid calibration unit (LCU). PTR-ToF-MS conditions were optimized in terms of inlet flow, inlet temperature, drift temperature, drift pressure and electric field. In order to reduce reactivity of aliphatic amines on the surfaces of the analytical system we carried out experiments at different temperatures and pH and using inert materials for LCU and PTR inlets.

Under standard conditions the high reactivity of aliphatic amines results in memory effects and large delay of responses. Using inert materials, high temperature and pH 7.4 we were able to analyse trimethylamine without any delay; for methyl- and dimethylamine delays were reduced. Under these conditions, calibrations showed good linearity with correlation coefficients > 0.99. LODs ranged from 0.8 ppbV to 27 ppbV and LOQs from 1.4 ppbV to 52 ppbV.

As the newly developed method reliably enables real-time determination of aliphatic amines in human breath, fast changes of normal and disease related metabolic processes in the whole body can be monitored online and non-invasively.
Breath analysis holds promise for non-invasive diagnosis. During breath sampling, subject’s physiology (breathing patterns or posture) affects VOC exhalation and may override true pathophysiological impression. Sampling standardization is thus crucial. As paced and spontaneous breathing are often used for sampling, we applied real-time mass spectrometry to investigate their effects onto VOC exhalation.

After having ethical approval and signed informed-consent, we applied an online PTR-ToF-MS-8000 [PDrift=2.3mbar, TDrift=75°C, VDrift=610Volt, E/N=139Td, Time-resolution=200ms] for breath-resolved VOC measurements in 20 healthy humans during continuous side-stream sampling [flow=20ml/min]. Oral breathing pattern was changed after every 3 min; i.e. spontaneous→paced (12breath/min)→spontaneous. Custom “Breath tracker” algorithm was used to assign alveolar and inspiratory concentrations of VOCs. Simultaneously, we performed continuous spirometry and capnometry.

Ventilation altered instantly, which thereby changed VOC exhalations. During paced breathing, tidal-volume and minute-ventilation increased by 35% and 25%, respectively and end-tidal PCO2 gradually decreased by 10%. Alveolar isoprene concentrations decreased significantly mirroring CO2 exhalation. Exogenous furan closely mirrored isoprene. Despite increased minute-ventilation, endogenous acetone remained unaffected. Oral H2S, gut originated C2H6S mirrored acetone. Smoking related acetonitrile and environmentally accumulated benzene and limonene also remained constant. All measured parameter returned to initial level during the final phase of spontaneous breathing.

Changes in breathing patterns immediately affected exhaled VOC concentrations. These effects were compound specific and depended on respiratory parameters and physico-chemical properties of the VOCs. Reliable sampling must define either spontaneous or paced breathing before clinically interpreting VOC concentration changes as breath biomarkers.
Background: Breath analysis holds promise for noninvasive in vivo monitoring of disease related processes. However, the influence of physiological parameters on the composition of exhaled air affects concentrations of VOC profiles. Volatile substances may be released via the alveoli, the bronchial mucosa or from the upper airways. The aim of this study was the systematic investigation of the influence of different sampling sites in the respiratory tract onto VOC concentration profiles.

Methods: After ethical approval breath samples from 20 patients undergoing bronchoscopy for various reasons were collected from different sites in the airways. For each position in the respiratory tract 30 mL breath gas were withdrawn by means of gastight glass syringes through the bronchoscope channel. 10 mL were used for VOC analysis; 20 mL for PCO2 determination. Samples were pre-concentrated by solid-phase micro-extraction and analyzed by gas chromatography-mass spectrometry. PCO2 was determined in a conventional blood gas analyzer. VOC concentrations were normalized onto the corresponding PCO2.

Results: After quantification and normalization changes in substance concentrations between different sampling sites were observed. Acetone concentrations were 16% higher in proximal sites when compared to more distal sampling sites. Isoprene concentrations decreased by 9% when passing from distal to proximal sampling sites. Isopropanol as external disinfectant showed high levels in room air but remained low in patients’ respiratory tract. Blank bronchoscope measurements did not show any contaminations.

Conclusion: Increased concentrations in the upper respiratory tract may be explained through substance excretion from bronchial mucosa while decreased concentrations could result from adsorption. Mapping of VOC profiles may provide novel insights into substance specific exhalation kinetics and mechanisms.

Keywords: Biomedical, GC-MS, SPME, Volatile Organic Compounds
Application Code: Biomedical
Methodology Code: Gas Chromatography/Mass Spectrometry
Quantitative Determination of Pteridine Levels in Urine Samples by Using HPLC-MS/MS for Potential Early Bladder Cancer Screening

Pteridines are metabolic intermediates used in the synthetic pathways of multiple cofactors and vitamins. Their integral role in these biosynthetic pathways is what makes pteridines such a good candidate for disease biomarkers. Used as biomarkers, two types of pteridines are of main focus: GTP derived and folate derived. Research has shown GTP derived pteridines have function in infections, autoimmune disease, and most importantly, cancer. The role of pteridines in cancers is the primary goal of this project. Folate derived pteridines are becoming an increasingly studied risk biomarker for multiple cancer types. For instance, studies have shown women with breast cancers have been reported as having higher levels of pteridines found in their urine. This project expanded upon the pteridine studies and investigate their roles within bladder cancers. This study compared the pteridine levels in urine and blood samples that have been collected from both healthy patients and patients suffering from bladder cancers. After pretreatment of the samples, they were analyzed using an HPLC-MS/MS method developed for the detection of pteridines. The pteridine levels for both healthy and cancer samples were determined. The detailed experimental procedures, the results, and the data on correlations of pteridine levels in urine and blood samples with the development of bladder cancers will be presented at the conference. This project moves forward into implementing these biomarkers in a way to more efficiently screen for bladder cancers in patients.

Keywords: Bioanalytical, Biomedical, Liquid Chromatography, Mass Spectrometry
Application Code: Biomedical
Methodology Code: Liquid Chromatography/Mass Spectrometry
The study of volatile organic compounds (VOCs) released by a biological system, so called volatolomics, is a promising non-invasive and fast diagnostic tool. The VOC profile is affected by physiological processes and therefore reflects the status of the cellular metabolism, like a ‘fingerprint’. The volatile characterization of metabolic changes triggered by cancer associated mutations will give clinically valuable information on prognosis and therapy response. So far, VOC analysis in clinical environment is still limited due to the lack of standardized procedures and reliable as well as predictive, diagnostic and prognostic biomarker compounds. This study was aimed at investigating the composition of VOCs released from in vitro cultured cancer cells for the identification of compounds produced or consumed directly by cancer cells and their microenvironment. Therefore, the head space of cell culture medium from three different cancer cell types was sampled onto thermal desorption tubes and analyzed by highly sophisticated two-dimensional gas chromatography coupled to high resolution time-of-flight mass spectrometry (GC×GC-HRTOFMS). Various univariate and multivariate statistics, such as analysis of variance (ANOVA), principle component analysis (PCA) and ANOVA simultaneous component analysis (ASCA), were compared for the interpretation of the high complex data set obtained and subsequent discrimination of the different cancer cell types. Volatile characterization and identification were done by mass spectral interpretation of the spectra.

The research was supported by Wallonie-Bruxelles International.

**Keywords:** Biological Samples, Data Analysis, Gas Chromatography/Mass Spectrometry, Volatile Organic Compounds
The synthesis of porphyrin polymers or porphyrimers provides the potential for diverse applications in several areas of life and industry. Most notable are the formulation of artificial blood, and the development of photocatalysts for solar energy conversion, development of electronic conductors and semi-conductors, development holographic image devices, photodynamic therapy and energy storage. Even after decades of research much of these applications are yet to be realized. Hence, we attempted to design and develop a new porphyrin polymer that maybe capable of particular application as artificial blood. In this study, we present the synthesis and characterization of tetra-a,b,g,d-3,5-diaminophenylporphyrin and it metal analog, tetra-a,b,g,d-3,5-diaminophenylporphyrinatoiron(II). The polymerization process will be achieved using known amide reaction techniques employing diacid chlorides such as oxaloyl chloride as the co-monomer. Various analytical techniques (uv-vis absorption and emission spectroscopy, GS-MS, LC-MS,NMR and elemental analysis) will be used for characterization of reaction product and intermediates.
Biomedical and Bioanalytical Electrochemistry

[i]In Vivo[/i] Detection of Oxidative/Nitrosative Stress Markers in Zebrafish Embryos

The reactive oxygen/nitrogen species (ROS/RNS) interplay is known to have both beneficial and destructive action in living organisms. However, the detection of oxidative/nitrosative stress markers is still a great challenge due to their low concentration, high reactivity, and short life-time in biological settings. Electrochemical approaches were shown to have several advantages over conventional ROS/RNS detection methods, with potential for application in the biomedical field. This presentation covers our latest results in the development and use of electrochemical microelectrodes for [i]in vivo[/i] detection of nitric oxide (NO) and hydrogen peroxide (H₂O₂) as representative markers of nitrosative and oxidative stress respectively. The performance of the microsensors is demonstrated by the use of zebrafish ([i]Danio rerio[/i]) embryos as a biological model. ROS/RNS concentrations are measured at organ level in live embryos, without additional perturbation beyond electrode insertion. The electrochemical measurements are cross-validated by the use of fluorescence imaging and specific pharmacological manipulation. Our results demonstrate the potential of electrochemical techniques to generate information about oxidative/nitrosative stress markers in biological settings.

Abstract Text

Keywords: Biomedical, Electrochemistry, Microelectrode
Application Code: Biomedical
Methodology Code: Electrochemistry
Applying a current through a synthetic membrane is used to enhance the delivery rate of ionic drugs. However, a precise quantification of the amount of drug delivered requires knowing what fraction of that current is carried by the drug ion. We have proposed a new way to do electrochemically induced membrane drug delivery. An ion selective membrane was used to transport our surrogate drug ion to a receiver solution while applying a voltage across the membrane. We have shown experimentally, and proven theoretically, that our membrane drug delivery prototype turns itself off when a quantifiable amount of drug has been delivered. Using this smart membrane, we have also found that the amount of drug delivered is precisely quantifiable. Finally, we have investigated the exponential dependence between the amount of drug delivered and applied voltage in accordance with the Nernst equation. We have concluded that small voltages, below 100 mV, are required to deliver around 90% of the drug from the feed solution.

This work was supported by the Nanostructures for Electrical Energy Storage (NEES), an Energy Frontier Research Center funded by the U.S. Department of Energy, Office of Science.
We have developed an analytical technique to measure bacterial metabolism in a simulated bacterial biofilm. Using a 3D inkjet printing technique we were able to print [i]Streptococcus mutans[/i] bacteria microhabitats using a newly synthesized biocompatible dendron-based hydrogel. We were able to build bacteria microhabitats of different sizes and able to position them at different distances from each other. In this study we have proved the bacteria are viable within the bulk hydrogel and also in the microhabitat. We have also developed a fast responsive solid state pH ultra-micro sensor which shows Nernstian slope of 58±2 mV/pH to be used as chemical probe in scanning electrochemical microscopy (SECM). Bacteria distribution was characterized within the bacterial microhabitat using fluorescence confocal microscopy using GFP tagged [i]E. Coli[/i] and found to be distributed homogenously within the microhabitat. Further the morphology of the bacteria microhabitat was characterized by using confocal microscopy and SECM. The recent findings about the change in local surrounding the microhabitat using newly developed solid state pH ultra-micro sensors will be presented in the meeting.

Keywords: Bioanalytical, Electrochemistry, Potentiometry
Application Code: Bioanalytical
Methodology Code: Electrochemistry
Recently, there have been significant advancements in electrochemical biosensors, many of which are defined by a DNA monolayer floor that employs either hybridization-based sensing or probe-target binding to induce a signal change. However, most are customized to a particular target and are thus restricted to analyzing, for example, either small molecules or proteins. In this work, we designed a DNA-based nanostructure capable of quantifying a range of targets. While most sensors operate via a distance dependent change, our nanostructure design is based on a fixed-distance redox reporter that reports mainly the diffusional changes to a binding region near the reporter. The DNA nanostructure was assembled on the electrode surface through enzymatic ligation with T4 DNA ligase (Figure), which significantly reduces costs compared to solid phase synthesis of the entire nanostructure. To operate in small molecule quantification mode, a larger binding partner is used as a “diffusion anchor.” A small molecule target is tagged to the binding region, followed by the introduction of a large anchor molecule which specifically binds with the target. When a sample is introduced, it displaces the large molecule “anchor,” which increases the diffusion coefficient of the redox tag and therefore increases square-wave voltammetry (SWV) current. This method is therefore an indirect, signal-ON small molecule sensor. To quantify protein, a binding partner for the target protein is covalently linked to the DNA nanostructure, and the protein target directly decreases the diffusion coefficient, thereby operating as a direct, signal-OFF protein sensor. This system was successfully validated by using desthiobiotin (kd= 10^-11 M with streptavidin) as the binding partner, allowing detection of either biotin (small molecule) or streptavidin (protein), as shown in the figure. This system should be applicable to a range of target molecules to simplify biosensor design using SWV.

Keywords: Bioanalytical, Biosensors, Electrochemistry, Voltammetry
Application Code: Bioanalytical
Methodology Code: Electrochemistry
Anion gap (AG) is an important parameter for the clinical diagnosis of various diseases and conditions. It provides information on the acid-base disorder (acidosis and alkalosis), which is caused by a number of conditions. AG refers to the difference in concentration between total measured cations (usually Na+ & K+) and total measured anions (Cl- & HCO3-) in serum, plasma or urine, expressed in millimolar (mM).

\[
AG = ([\text{Na}^+] + [\text{K}^+]) - ([\text{Cl}^-] + [\text{HCO}_3^-])
\]

As shown in the equation, AG is not a directly measurable quantity. It is calculated from separate test results of the measurable ions listed above and is prone to errors. We present here a simple and fast method for the determination of AG by simultaneous measurement of total measurable cations and total measurable anions using pulsed chronopotentiometry (pulstrode) with ionophore-free polymer membrane ion-responsive electrode. The membrane used here is void of ion-exchange property and is doped with a neutral lipophilic salt of the form R+ R-, where R+ and R- are a lipophilic cation and a lipophilic anion, respectively. Current pulses are applied via a bipotentiosstat to cause polarization of the membrane surface. The current is scanned in the positive (anodic) and negative (cathodic) directions. When an anodic current is applied the lipophilic cation (R+) migrates to the sample/membrane interface and allows measurement of the anions. When a cathodic current is applied the lipophilic anion (R-) migrates to the sample/membrane interface and enables measurement of the cations. Thus, the total measurable cations and the total measurable anions can be measured under cathodic current and anodic current, respectively, and the AG can be calculated.
Biomedical and Bioanalytical Electrochemistry

Electrochemical Immunosensor: CNT Modified Hand-painted Sensors for Amperometric Detection of Cancer Biomarker

Point-of-care diagnostics entail development of methods, devices, and materials to build ready-to-use tools for rapid and sensitive detection of diseases at patients’ bedside. Current methods of device fabrication are laborious, expensive, and require special skills. These disadvantages make healthcare tools inaccessible to resource deprived population of the world. In this poster, we show development of the simplest, inexpensive and sensitive electrochemical immunosensor that requires minimum technical skills and hardware. Briefly, patterns of three-electrode system were printed using wax printer (Xerox ColorQube 8580) on polyethylene terephthalate (PET) substrate followed by hand painting of conductive silver layer using 30 wt% Ag ink in the hydrophilic spaces. After sintering, working electrodes were modified by dropping multiwalled carbon nanotubes (MWCNTs) followed by curing at 120 C. These sensors were electrochemically characterized by voltametric and amperometric techniques for several figures of merit including dynamic range, limit of linearity, analytical sensitivity, and calibration sensitivity. A magnetic bead based immunoassay was employed to detect carcinoembryonic antigen (CEA), a colon cancer biomarker, by amperometry. The normal level of CEA in blood is less than 5 ng/mL, while higher levels is considered to be a risk factor. The limit of detection for CEA was 0.6 ng/mL using 1.0 mm diameter working electrodes, which is significantly lower than the clinical cutoff value. Moreover, cost of the CNT modified hand-painted chip is 100 times lower than the counterpart commercial screen-printed electrodes. We propose that the CNT modified hand-painted sensors will allow routine bioassays to perform at very low cost.

Keywords: Bioanalytical, Electrochemistry, Electrode Surfaces, Immunoassay
Application Code: Bioanalytical
Methodology Code: Electrochemistry
Zebrafish (Danio rerio) are a subtropical teleost, originally developed as a research model of development, that has recently found use as a model of neurological function. Here, we have used fast-scan cyclic voltammetry at carbon fiber microelectrodes as well as imaging techniques to characterize the release process of dopamine, a neurotransmitter implicated in a wide range of brain functions including reward and cognition. In particular, we examined the release and uptake of dopamine ex vivo in zebrafish whole brains in response to chemotherapeutic agents such as carboplatin and 5-fluorouracil. We also will discuss measurements of dopamine released by remote electrical stimulation of the neuronal pathway.

Keywords: Bioanalytical, Electrochemistry, Microelectrode, Voltammetry
Application Code: Bioanalytical
Methodology Code: Electrochemistry
Extracellular glucose detection is critical to understand glucose metabolism in human erythrocytes and blood sugar regulation. Until now, most of electrochemical glucose measurement methods are developed with functionalization of glucose oxidase (GOD) on electrode surface. Glucose can be measured by monitoring electrons flow through electrode. However, limited by the electrode surface area, only bulk glucose concentration can be measured. Patch clamp provides an alternative method for localized small amount chemical detection. In this technique, glass micropipettes are pressed into cell membrane to get a patch of membrane. With access to ion channels or transporters on membrane, electrochemical properties of these proteins can be studied and applied for selective chemical detection. Herein, we applied this technique to design a transporter probe for glucose measurement. Sodium-glucose linked transporter 1 (SGLT1) is a type of cotransporter in which two sodium cations would be transported with one glucose molecule. With this property, glucose concentration can be correlated with sodium cation concentration and then be simply measured by the current change through membrane.
Saccharomyces cerevisiae yeast cells are used as a model organism to investigate the effects of various pulsed electric fields on cell wall permeability to different compounds and to propose the mechanism of permeabilization. As known, cell wall of Saccharomyces cerevisiae is the selective barrier for entering the essential cell nutrients and minerals; the cell wall and membrane limit uptake of small hydrophilic as well as restrict the velocity of big lipophilic molecules uptake. The tetraphenylphosphonium cation was used for the analysis of the lipophilic compounds uptake dynamics and of cell wall permeabilization mechanism. The TPP+ absorption process was analyzed applying different pulsed electric field treatment regimes in corresponding medium and using a potentiometric ion selective electrode and a minipotentiostat designed for mobile use with the custom build firmware. The possible yeast cell wall permeabilization mechanism calculated mathematically and based on empirical results will be proposed, as well as the compensation solution for non-linear sensor response will be discussed.
Platelets are blood borne anuclear circulating bodies commonly known for their role in clot formation during vascular injury to prevent blood loss. In the event of injury, platelets migrate to the injured site and secrete chemical messengers, during a process known as exocytosis, to recruit immune cells including additional platelets to initiate clot formation. Additionally, platelets play an essential role in a variety of diseases, including malaria. Malaria is a worldwide epidemic with the highest infection rates in developing countries, including much of Africa. The infection occurs by the transmission of Plasmodium parasites through the bite of infected female mosquitoes. Currently, there is no vaccine available due to continuous parasite resistance to anti-malarial drugs. The role of platelets in malaria is unclear, with some studies finding platelets helping to fight off malaria while others find platelets to facilitate the transfer of the parasites. The work herein involves the use of the electrochemical technique, carbon-fiber microelectrode amperometry (CFMA), to experimentally characterize electroactive serotonin (5-hydroxytryptamine, 5-HT) that is secreted from platelets to understand how platelet release dynamics are affected in the context of malaria as well as in the presence of common anti-malarial drugs such as chloroquine and pyrimethamine. Results reveal that platelet dynamics depend largely on the stage of malarial infection, whereas the studied anti-malarial drugs have no effect on platelet mechanical released of 5-HT.

Funding acknowledgement:
Diversity of Views and Experiences Fellowship
Biotechnology Training grant (NIH T32GM00847)

Keywords: Biomedical, Electrochemistry, Single Molecule
Application Code: Biomedical
Methodology Code: Electrochemistry
Biomedical and Bioanalytical Electrochemistry

Accurate Simulation of Electrochemical Problems of Any Complexity

KISSA-software (for 1D and 2D microelectrode geometry) developed in our group, provides a general framework to treat electrochemical problems (by providing mechanism, rate constant, diffusion coefficients etc.) of any complexity in a user-friendly environment and returns the simulations results without any intervention into numerical part from the user side [1]. The accuracy of the numerical solution is guaranteed in KISSA by employment of a non-uniform and adaptive grid. The latter is constructed on the basis of a kinetic criterion (rather than on a gradient-based one as in other programs) and provides a high dynamic resolution at the acute reaction fronts which are automatically detected and followed by the program. The efficiency of this strategy was proved by addressing such sophisticated problems as i) simulation of reaction mechanisms leading to the emission of electrochemiluminescence (ECL) and ii) including the reactive dynamic adsorption. In this context considered ECL systems are representative example of systems possessing extremely sharp reaction fronts since some reaction constants are close to the diffusion limit (either cation/anion radicals annihilation reactions or luminophor/co-reactant reaction). More precisely, for the ECL co-reactant system, such as alkyl amines / transition metal(II) complexes, it was shown via simulations with KISSA that changes in ECL intensities emitted by these systems are much more dependent on the relative diffusivities of the two co-reactants than on the range of thermodynamic and kinetic rate constants that are possible to explore and vary. Second example is the competitive reactive adsorption is also involved as an important class of problems covered by KISSA. A couple examples of real data imported to KISSA for comparison to simulations for elucidation of mechanism and determination of the parameters are presented.

Abstract Text

KISSA-software (for 1D and 2D microelectrode geometry) developed in our group, provides a general framework to treat electrochemical problems (by providing mechanism, rate constant, diffusion coefficients etc.) of any complexity in a user-friendly environment and returns the simulations results without any intervention into numerical part from the user side [1]. The accuracy of the numerical solution is guaranteed in KISSA by employment of a non-uniform and adaptive grid. The latter is constructed on the basis of a kinetic criterion (rather than on a gradient-based one as in other programs) and provides a high dynamic resolution at the acute reaction fronts which are automatically detected and followed by the program. The efficiency of this strategy was proved by addressing such sophisticated problems as i) simulation of reaction mechanisms leading to the emission of electrochemiluminescence (ECL) and ii) including the reactive dynamic adsorption. In this context considered ECL systems are representative example of systems possessing extremely sharp reaction fronts since some reaction constants are close to the diffusion limit (either cation/anion radicals annihilation reactions or luminophor/co-reactant reaction). More precisely, for the ECL co-reactant system, such as alkyl amines / transition metal(II) complexes, it was shown via simulations with KISSA that changes in ECL intensities emitted by these systems are much more dependent on the relative diffusivities of the two co-reactants than on the range of thermodynamic and kinetic rate constants that are possible to explore and vary. Second example is the competitive reactive adsorption is also involved as an important class of problems covered by KISSA. A couple examples of real data imported to KISSA for comparison to simulations for elucidation of mechanism and determination of the parameters are presented.

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<tr>
<td>Primary Author</td>
<td>Irina Svir</td>
</tr>
<tr>
<td>Author</td>
<td>CNRS</td>
</tr>
<tr>
<td>Co-Author(s)</td>
<td>Alexander Oleinick, Christian A. Amatore</td>
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Graphene, one of the most attracting two-dimensional nanomaterials, has demonstrated a broad range of applications because of its excellent electronic, mechanical, optical and chemical properties. In this work a general, environmental-friendly, one-pot method for the fabrication of reduced graphene oxide (RGO)/metal (oxide) (e.g. RGO/Au, RGO/Cu2O, and RGO/Ag) composties was developed using glucose as the reducing agent and the stabilizer. The glucose not only reduced GO effectively to RGO, but also reduced the metal precursors to form metal (oxide) nanoparticles on the surface of RGO. Moreover, the RGO/metal (oxide) composites were stabilized by gluconic acid on the surface of RGO. The developed RGO/metal (oxide) composites were characterized using STEM, FE-SEM, EDS, UV-vis absorption spectroscopy, XRD, FT-IR and Raman spectroscopy. Finally, the developed nanomaterials were successfully applied as an electrode catalyst to simultaneous electrochemical analysis of L-ascorbic acid (L-AA), dopamine (DA) and uric acid.

Keywords: Bioanalytical, Electrochemistry, Nanotechnology

Application Code: Bioanalytical

Methodology Code: Electrochemistry
The simultaneous detection of multiple species in environmental and clinical analysis is often laborious, time consuming, and requires expensive instrumentation. Multiplexed detection is important nonetheless, as most samples contain a mixture of species of interest. Multiplexed paper-based analytical devices (PADs) have been developed to circumvent this problem, providing portable, inexpensive, automated and user friendly analytic measurement platforms, with electrochemical or colorimetric detection particularly common. Multiplexed colorimetric PADs are common, but suffer from poor sensitivity. Very few multiplexed electrochemical PADs (ePADs) have been developed. In this work, norepinephrine and serotonin are detected simultaneously, at the optimal pH conditions of each species with an ePAD. It is possible to alter pH conditions \textit{in situ} via impregnation of paper channel zones with an acid or a base to decrease or increase the solution pH reaching the electrochemical detection zone (Figure 1-i). Dual pH measurements are also demonstrated with p-aminophenol, to enable quantification of \{beta\}-galactosidase enzyme kinetics in a dual pH system (Figure 1-ii), as well as monitoring of multiple enzymes simultaneously. Boron doped diamond paste electrodes (BDDPE) are developed, optimized, and interfaced with the dual pH ePADs, enabling low limits of detection as a result of BDD's low background currents compared to conventional electrode materials.\textsuperscript{1} These BDDPE-ePADS allow for low-cost, sensitive, and selective electrochemical experiments to be carried out in multiple solution conditions on a single sample simultaneously for the first time, further automating and simplifying electrochemical analyses.


**Keywords:** Electrochemistry, Electrodes, Enzyme Assays, On-line

**Application Code:** General Interest

**Methodology Code:** Electrochemistry
In the present research, the scanning electrochemical microscopy was applied for the investigation of bioelectrochemical activity. The scope of this work was to use Saccharomyces cerevisiae and Aspergillus niger as the biocomponents in the mediated electrochemical biosensors. Due to the fact that fungi are eukaryotic organisms, a lipophilic mediator was necessary for the interaction with the intracellular redox molecules associated with the catabolism[1]. The principle action of the double redox mediators based system, which has been applied in this research, is demonstrated in Fig.1. The mediators act as electron carriers in a chain of reactions, which link intracellular activity with the medium outside the cell, resulting in the current that is registered by an Ultra Micro Electrode. We examined the detection of the catabolism in two microorganisms before and after their modification with a conductive polymer- polypyrrole. The results indicated that, by encapsulating Saccharomyces cerevisiae with the polypyrrole, the signal decreased by increasing the concentration of the pyrrole in which the cells were incubated. On the opposite side, the encapsulation of Aspergillus niger cells with polypyrrole enhanced amperometric and scanning electrochemical microscopy signals by several times. It could be due to improved charge permeability or a better conductive cell wall. Both these changes appeared to boost sensitivity in biosensors or on the other hand increase the power density in microbial fuel cells, due to both being associated to current density. This improvement in the electron transfer could be used for new methods of improving biosensors or microbial fuel cell electrochemical systems.


Keywords: Biosensors, Biotechnology, Electrochemistry, Microelectrode
Application Code: Bioanalytical
Methodology Code: Electrochemistry
Here we report a new method to measure the rate of 4-nitrothiophenol (NTP) reduction on bimetallic alloy surfaces. First, using bipolar electrodeposition techniques, we formed Ag/Au alloy films on stainless steel substrates in which the molar ratio of Ag varied continuously from around 50% to 100%. Self assembled monolayers NTP formed on these surfaces were characterized as a function of time an applied potential in a bipolar electrochemical cell using confocal Raman microscopy. The rate of reduction of NTP was shown to be dependent on the alloy composition. Specifically, our results show that an alloy composition that is approximately 50% Ag is optimal.

**Keywords:** Characterization, Electrochemistry, Electrode Surfaces, Infrared and Raman

**Application Code:** Bioanalytical

**Methodology Code:** Electrochemistry
Platinized carbon nanoelectrodes with well-characterized geometry were used as scanning electrochemical microscopy (SECM) tips to measure ROS and RNS (hydrogen peroxide, peroxynitrite, nitrogen monoxide and nitrite ion) inside non-cancerous and metastatic human breast cells. By performing time-dependent quantitative amperometric measurements at different potentials, in the cell cytoplasm and their dynamics were determined and used to elucidate the chemical origins and production rates of ROS/RNS in non-transformed and metastatic human breast cells. In addition to cytoplasm detection, these small and sharp probes offer a means to quantify ROS/RNS in subcellular compartments, including phagolysosomes. The previously developed approach based on the four-potential pulse program was used to measure relative concentrations of four key ROS/RNS inside a single phagolysosome in an IFN-γ and LPS stimulated macrophage. The results can contribute to the understanding of the origins of inflammation and immune system diseases.
Pyocyanin has a promising future as a biomarker for the identification of Pseudomonas aeruginosa, an opportunistic pathogen that can cause life-threatening diseases. Pyocyanin is an electro-active molecule that can be oxidized at a potential of -250 mV vs. Ag/AgCl. This feature enables the detection of pyocyanin by electrochemical approaches, which are more rapid, cost-effective, and easier to implement than detection of the bacterial cells. Thus far, pyocyanin has been detected electrochemically using carbon fiber,[1] micro-fabricated Au electrode,[2,3] and commercially available carbon electrodes.[4] However, compared to smooth planar electrodes, vertically aligned nanowire array electrodes exhibit even higher sensitivity and have been widely used as biosensors due to their large surface area. For example, it is reported by Jamal, et al.[5] that the sensitivity of glutamate examined by a nickel nanowire electrode increased roughly 279 times compared against a flat Ni electrode.

Herein, we demonstrate the fabrication of metal alloy nanowire electrodes by electrodeposition of material into anodic alumina oxide (AAO) templates with a nominal pore size of 200 nm. The composition and morphology of vertically aligned nanowire array electrodes is characterized by X-ray fluorescence (XRF) and scanning electron microscope (SEM) respectively. Electrochemical detection of pyocyanin on the nanowire electrodes is performed by square wave voltammetry (SWV).

[b]References[/b]

Keywords: Biosensors, Electrochemistry, Electrode Surfaces, Sensors
Application Code: Bioanalytical
Methodology Code: Electrochemistry
Gold is among the most used materials in electrocatalysis and electroanalysis but this noble metal is still too expensive to be used in the fabrication of low cost and disposable devices. In the present work, gold-leaf sheets, usually employed in decorative crafts and wedding candies, is introduced as an inexpensive source of gold for electrodes by simply and easily gluing them onto Kapton polyimide tape.

Planar-disc and nanoband electrodes were manufactured by sandwiching the gold leaf between two strips of polyimide tape, one shorter than the other. The exposed area of gold at one end was reinforced with silver ink, thus forming a terminal for electrical contact. By cutting the edge at the other end of the sandwich with a razor blade, the nanoband electrode was obtained. The exposed area (3mm diameter) of the Au disc electrode was defined by a hole previously punched in one of the strips with a paper punch. A Pt wire (1mm diameter) and Ag/AgCl(KCl 3M) electrode were used as auxiliary and reference electrodes, respectively, in all cyclic voltammetry (CV) experiments.

The planar disc electrode exhibited electrochemical behavior similar to that of a commercial gold electrode in 0.2M H2SO4. The CV of a 1mM solution of potassium ferricyanide in 0.2M KNO3, presented oxidation and reduction peak potentials that where 80mV apart. The electrode also offers promising prospects for the development of wearable devices. When submitted to severe mechanical deformation, this electrode exhibited neither loss of electrical contact nor significant variation in voltammetric response, even after fifteen bending and folding cycles. The circa 200nm thickness of the gold-leaf sheet favors the production of nanoband electrodes with behavior similar to that of ultramicroelectrodes. The electrode surface can be easily and reproducibly renewed by cutting a thin slice off its end with a razor blade; the CVs present limiting currents with a relative standard deviation (RSD) of 3.8% (n=5).

Keywords: Electrochemistry, Electrodes, Microelectrode, Voltammetry
Application Code: Bioanalytical
Methodology Code: Electrochemistry
In gas chromatography, inert surfaces in the flow path from the inlet through the column to the detector are essential for achieving good peak shapes, low level detection and accurate results for active analytes. Active analytes that stick to exposed silanols and other chemically active sites in the flow path include: organic acids and bases, sulfur species, alcohols, amines, aldehydes, phenols, and pesticides. Accurate and reproducible measurements of these analytes play important roles in international commerce, product quality, environmental preservation, and human health risk assessment for consumer products and foodstuffs. Modern GC and GC/MS instruments are capable of routinely monitoring single digit part per billion (PPB) levels of analytes provided the analytes survive the trip from sample injection, vaporization in the inlet, separation on the column and delivery to the detector. Assuring that analytes survive the trip depends in large part on the quality of the consumable products in the flow path that come in contact with the sample. Example chromatograms of active analytes using inert flow path consumables compared with less inert consumables show visually why inertness matters. A flow path diagram is a very useful tool in understanding where and how a lack of inertness can negatively impacts chromatography. Remedies for avoiding potential pitfalls in the various sections of the flow path are displayed with a flow path diagram as the backdrop.

Keywords: Capillary GC, Environmental/Soils, Gas Chromatography/Mass Spectrometry, Semi-Volatiles
Application Code: High-Throughput Chemical Analysis
Methodology Code: Gas Chromatography/Mass Spectrometry
Introduction
Bacteria emit a broad spectrum of volatile substances. In a perspective VOC patterns may therefore help to recognize bacterial growth and infections non-invasively. This study was intended to characterize VOC profiles from Streptococcus pyogenes cultures with respect to proliferation phases.

Method
20 ml of the headspace above bacterial cultures were sampled bidirectionally 1, 4, 5, 6, 7, 10, 24, 48 and 72 hours after inoculation onto polymer needle trap devices (NTDs). VOCs were thermally desorbed from NTDs and further analysed by GC-MS. Bacterial growth was assessed through determination of optical density of bacterial cultures. At every time point, headspace of three bacteria cultures were sampled. In parallel three media samples were taken as control.

Results
VOC profiles mirrored growth kinetics. Some VOCs were only detectable in the headspace of S. pyogenes and not in the medium. The heatmap indicates that some aldehydes, alkanes, pyrazines accumulated after 5 and 6 hours. After this maximum, concentrations of most substances decreased again. Most alcohols and ketones reached their maximum after 72 hours. First accumulation of VOCs takes place when growth of Streptococcus pyogenes changes from exponential to stationary growth phase.

Discussion
Time course of VOC patterns mirrored active adjustments of metabolic pathways during bacterial proliferation. In addition, VOC determination may reveal metabolites having toxic or virulence associated properties. VOC analysis could be used for non-invasive monitoring of growth and metabolism in bacterial cultures.

Keywords:
- Gas Chromatography/Mass Spectrometry
- GC-MS
- Headspace
- Volatile Organic Compounds

Application Code:
- Bioanalytical

Methodology Code:
- Gas Chromatography/Mass Spectrometry
Conventional GPC analysis has the ongoing issue of long analysis time and high-volume solvent usage. In order to solve these problems, Shodex introduced a rapid analysis GPC column, GPC HK-404L, using a porous cross-linked styrene-divinylbenzene polymer gel with sharp particle size distribution. The HK-404L can be used for molecular weight measurement with the range from 100 to 1 million Daltons. We have recently developed two new columns, one for lower molecular weight oligomers analysis in the molecular weight range from 100 to 1,500 Daltons and another column for higher molecular weight polymer with molecular weight from 10,000 to 2,500,000 Daltons. In both cases, the elution volume with less than 2.0 mL. Additionally, basic features of these novel GPC columns and several cases of molecular weight measurement will be presented.
We have shown that a “green” mass spectrometry compatible liquid chromatography method using a Brij-35 modified C18 UHPLC column with no surfactant in the water mobile phase can separate di-carboxylic (terephthalic, isophthalic, phthalic) and tri-carboxylic (trimesic, trimellitic, and hemimellitic) acids, all in the same mixture. Using a flow gradient, this isocratic separation took only 10 min with baseline or near baseline resolution. Using a standard C18 reversed phase approach at low pH, resolution of such polar compounds is lost. The retention mechanism is likely due to the interaction of the aromatic acids with the (CH\[sub\]2[/sub\]CH\[sub\]2[/sub\])\[sub\]23[/sub\]OH chain of Brij-35; the C12 hydrocarbon chain of the surfactant is hydrophobically bound to the C18 stationary phase. Currently we are exploring the nature of this retention mechanism using a variety of polyoxyethylated surfactants. To test the generality of Brij modified stationary phases for this separation, Brij-58 with a (CH\[sub\]2[/sub\]CH\[sub\]2[/sub\])\[sub\]20[/sub\])OH group with a cetyl (C16) hydrocarbon chain is being tested. Polysorbate surfactants such as Tween 20 and Tween 80 are also of interest. This class of non-ionic surfactants has four polyoxyethylated chains (n=20) with a variable hydrocarbon ester chain such as C18 for Tween 80 and C12 for Tween 20. The expected increase in the polyoxyethylated chain density should markedly affect retention. Column temperature may become an important experimental factor to consider.
The combination of the antiretroviral drugs emtricitabine and tenofovir disoproxil is used to treat HIV patients. The impurity profiling of the cocktail drug by reversed phase is difficult due to the co-existence of early-eluting polar and hydrophobic impurities. A ternary reversed phase gradient was previously developed to profile the impurities of the cocktail drug within 10 minutes. The method relied on UV detection. Quantitation of the two active pharmaceutical ingredients (API) can only be achieved by calibration with two standards at different levels, due to the extinction coefficient difference. The Charged Aerosol Detector (CAD) is regarded as universal detector, thanks to very similar response to different non-volatile molecules. This means that, in principle, quantitation of different entities can be achieved by drawing just one calibration curve obtained from one of the components to be analyzed. However, even if independent from the nature of the molecule, response depends on the solvent that needs to be nebulized. Consequently, response during a gradient will vary based on the mobile phase composition. An established practice in CAD with gradient chromatography is to apply an inverse gradient that will merge with the gradient delivered through the column at a point between the column outlet and the detector nebulizer. In this way, the composition of the effluent entering the nebulizer of the detector will be constant, and the detector response will remain stable. This approach has been extensively applied to binary gradients. In this work, the mixture of the antiretroviral drug is used to demonstrate the inverse ternary gradient in analysis of drugs based on multiple APIs. A novel UHPLC system was used for this purpose. The systems features a high precision dual pump capable of delivering two independent gradient with up to three components. The quantitation accuracy and sensitivity of the CAD with and without inverse gradient are compared.
Vitamins are essential nutrients in animal's diet. They are organic molecules, but cannot be synthesized by the organism, hence they must be assimilated with food. Human's vitamins are divided in fat-soluble and water-soluble vitamins. Vitamins are routinely quantified in food and food supplements. Both fat and water soluble vitamins can be analyzed by Reversed Phase (RP) chromatography. However, due to the different solubility, and massively different retention behavior, the simultaneous analysis with the same method is avoided. If samples containing both classes of vitamins have to be analyzed, two different methods have to be run with the same system one after the other, or run simultaneously with two separated systems. The first approach is time-consuming. The second approach requires availability of two instruments. It is possible to circumvent the space disadvantage of operating two systems, by adding to a standard HPLC system and additional pump, and configuring complex switching valves setups; however separations can be operated only sequentially with no gain in throughput. Moreover the incompatibility of the mobile phases used in the two methods, could cause issues, for instance salt precipitation.

In this work we introduce a novel approach to simultaneous analysis of fat and water soluble vitamins by RP, with two independent columns run by the same UHPLC system simultaneously. The newly designed UHPLC consists of a pump capable of delivering eluents to two separated flow paths, and an autosampler with two independent injection units and a shared well-plate compartment. The solution enables high-throughput vitamin analysis. Programming and operating the system is simple and the need of complex valve switching configurations is not required.
Pharmaceutical companies often follow current USP methods for the analysis of raw materials and finished products by using many current USP methods. For many USP HPLC methods, the conditions often consist of long runtimes and higher flow rates. Although the conditions are selected for HPLC columns, the methods are often capable of being run on lower dispersion, higher pressure systems such as UHPLC and UPLC systems, as long as the system suitability requirements are met. The USP has outlined in the USP General Chapter <621> acceptable method adjustments to scale isocratic methods to provide the same if not improved performance. These allowable adjustments include scaling particle size and column dimensions to maintain L/dp ratio, where L is the length of the column and dp is the particle size of the packing material, and adjusting the flow rate and injection volume accordingly.

In this study, the USP assay method for quetiapine fumarate, an anti-psychotic drug, will be evaluated on a wide range of systems including an HPLC, a UHPLC and a UPLC liquid chromatographic system. This isocratic method will be evaluated under the conditions as stated per the USP Monograph: Quetiapine Fumarate (USP40-NF35 Page 5939). After testing of the original method on all systems, the method will be scaled following the USP allowable changes. The results will demonstrate the ability to decrease run times and improve throughput by scaling a method to smaller particle size and column dimensions.

Keywords: HPLC, Liquid Chromatography, Pharmaceutical, Standards
Application Code: Pharmaceutical
Methodology Code: Liquid Chromatography
Renal cell carcinoma (RCC) or kidney cancer is the third most common cancer of the genitourinary system and in 2015 it accounted for an estimated 61,560 new cases and 14,080 deaths in the United States. RCC, with current medical treatment, is difficult to detect, costly and time-consuming.

The purpose of this research study is to develop a fast and sensitive method using High-performance liquid chromatography (HPLC) to separate four human physiological biomarkers. It was reported that quinolinic acid, 4-hydroxybenzoic acid and gentisic acid levels in urine can be differently expressed when a person suffering from RCC. In this project, an HPLC method was developed and validated to quantify these metabolites in urine. In this project creatinine was included as an analyte in order to account for the renal dilution of urine.

The optimum separation of these metabolites were obtained in two chromatographic conditions using Agilent XDB Eclipse C18 column. Analytical method validation parameters such as Limit of Detection (LOD), Limit of Quantification (LOQ), Linearity, Accuracy, and Precision were determined for each metabolite. This method offers several advantages including negligible consumption of sample, simultaneous detection of analytes and high speed analysis. Also this HPLC method can be more applicable to clinical settings compared to the previously published LC/MS/MS method, because HPLC is a commonly available and less expensive instrument than the LC/MS/MS. Finally, this method can be utilized to analyze urine samples in a detailed clinical study and have a statistical comparison between these metabolite levels in RCC and RCC free urine samples. The detailed experimental conditions and results will be presented at the conference.

Keywords: Bioanalytical, HPLC, Metabolomics, Metabonomics, Method Development
Application Code: Bioanalytical
Methodology Code: Liquid Chromatography
The globally rising demand for enhanced product quality control has repeatedly driven the need for higher sample throughput to achieve more analytical answers in shorter time in all aspects of modern industries and markets, from food safety to pharma QC and consumer healthcare and many more. UHPLC technology was already a leap ahead for throughput enhancement, offering shorter run times at the same or even better result quality than conventional HPLC. If, however, the sample pipeline persistently exceeds the analytical capacity of a UHPLC system, then this bottleneck can typically be eliminated only by adding more instrumentation. This enables the parallelized processing of more samples per time but at the cost of precious resources like bench space and budget.

The Thermo Scientific™ Vanquish™ UHPLC Dual LC technology addresses this conflict by combining two individual flow paths in the footprint of a single system. Each flow path consists of a pump, an injection unit, a column and a detector. These two flow paths can be operated independently so that this Dual LC technology doubles the number of assays in the same time.

This work exploits the capabilities of Dual LC technology by parallelizing the UHPLC analysis of soft drinks for sweeteners and preservatives. Based on key chromatographic parameters, the variability between the two flow paths is assessed against two separate LC systems. Doubling the number of analyses per bench space is seamlessly feasible, providing the same result quality as two independent systems but without the double cost of ownership.

Keywords: Food Safety, HPLC, Optimization, Sample Handling/Automation

Application Code: Food Safety

Methodology Code: Liquid Chromatography
Chirality is a geometric property of some molecules. A chiral molecule/ion is non-superimposable on its mirror image. The presence of an asymmetric carbon center is one of several structural features that induce chirality in many molecules. The mirror images of a chiral molecule/ion are called enantiomers or optical isomers. Individual enantiomers are often designated as either "right-" or "left-handed". The concept is of great practical importance because most biomolecules and pharmaceuticals are chiral. In fact, half of all drugs in use are chiral. Cyclofructans are cyclic oligosaccharides and have been recently found to be very efficient in separating isomers of primary amines especially in the polar organic non-polar modes. Other stationary phases include cyclodextrin and macrocyclic glycopeptide based columns that reproduce separations like crown ether or protein based stationary phase columns. By bonding these selective phases to superficially porous particle Agilent InfinityLab Poroshell 120 columns, highly efficient columns are created.

In this work, enantiomeric separation capabilities of four superficially porous based chiral columns are evaluated in a screening process using a set of racemic primary amines and other chiral compounds. Various mobile phase compositions on an Agilent 1290 Infinity II Method Development Solution System, chiral column/mobile phase and additive combinations are quickly evaluated.
A chiral HPLC analysis combined with time-dependent density functional theory (TD-DFT) calculations was used for the assignment of the absolute configuration (AC) of both enantiomers of the antihistamine drug chlorpheniramine. Circular dichroism and optical rotation based detections coupled to the chromatographic system (HPLC-CD-OR) permitted the online measurement of the CD spectra (right graph) and [alpha][sub]670[/sub] values (left graph), while TD-DFT at the B3LYP/TZVP//B3LYP/VDZ(P) and B3LYP/aug-cc-pVDZ//B3LYP/VDZ(P) levels of theory for CD and OR, respectively, delivered theoretical chiroptical properties in the gas and solution states. Both calculations were consistent in predicting the correct combination of CD spectra and [alpha][sub]670[/sub] sign and therefore permitted the absolute configuration (AC) assignment of the optical isomers. The AC proposed using this methodology was in agreement with a previous X-ray study.

Keywords: Chiral, Chiral Separations, Drugs, HPLC Detection
Application Code: Drug Discovery
Methodology Code: Liquid Chromatography
Comparison of Four Different C18 HPLC Columns with Different Particle Sizes and Lengths for the Optimization of UV Absorbing Engine Coolant Additives Analysis

The performance of four different C18 chromatography analytical columns with different lengths and particle sizes were compared for analysis of engine coolant additives such as benzotriazole (BTz), tolyltriazole (TTz), sodium benzoate and cinnamic acid using high performance liquid chromatography (HPLC) with UV-visible detector. Chromatographic parameters including retention time, resolution, limit of detection (LOD), limit of quantification (LOQ), number of theoretical plates and reduced plate height were evaluated and compared. In this work, the performance of 5µm particles of Kinetex and Acclaim C18 chromatography columns has been compared with new positive surface (PS) and polar C18 columns having 3µm particles for the analysis of above additives. The results show that in comparison with the traditional columns having dimensions 250 mm×4.6 mm; 5µm, shorter columns namely polar C18 and positive surface (PS C18) having dimensions 150 mm×4.6 mm; 3 µm allowed the reduction of the analysis time by 44.8% and 45.8% respectively without compromising on column performance. This leads to the reduction of the analysis expenses by reducing of organic solvents and time, and increasing the total number of analyses per day. Among two smaller columns, the resolution of positive surface C18 is found to be better as compared to polar C18 column though the analysis times remains almost same. The mobile phase CH3OH (70%): 0.5%H3PO4 in water (30%) was used at flow rate of 0.8 mL/min and the injection volume in all the case was 20.0 µl. The capabilities of these columns for analyzing engine coolant samples in glycol based aqueous media were evaluated.

Keywords: Analysis, HPLC Columns, Liquid Chromatography, UV-VIS Absorbance/Luminescence

Application Code: Fuels, Energy and Petrochemical
Methodology Code: Liquid Chromatography
Assessment of properties of multi-dimensional separation methods is often based on “box counting” using a one-dimensional peak capacity paradigm. However, additional information could be made available, beyond peak capacity, to supplement retention time data in detecting the presence of target analytes. For example, using two serially arranged column segments in LC, each with its respective UV absorbance detector (at different wavelengths), absorbance ratios [1] can be combined with retention times for significantly improving target compound identification. In this case, the “detection capacity” of the separation is a combination of the overall peak capacity with the additional detection identification information contained in the absorbance ratios. This paper extends Blumberg’s separation measure [2] to include other statistics measured during a separation to create a detection capacity. This extension requires the relaxation of two common assumptions used in peak capacity calculations: orthogonality and uniform distribution. When the separation column consists of two or more segments, serially arranged, the orthogonality assumption is more problematic because some of the critical variables of the separation process, such as polarity of the mobile phase, remain constant across column segments. Second, high and low absorbance ratios between different wavelengths would not be expected to vary uniformly across the spectrum. In this work, a simple model of conditional linear correlation is used to evaluate detection capacity in the presence of correlation. Detection capacity is then extended to include non-uniformly distributed retention times and absorbance ratios. The detection capacity calculations are illustrated for a serially arranged nano-flow LC system.

Liquid Chromatography

### Synthesis and Characterization of Hydrophilic-Lipophilic Balance Particles for Various Solid Phase Microextraction Sampling Devices

Hydrophilic-Lipophilic Balance particles (HLB) have emerged as a promising material during the last two decades. Their ability to extract compounds of various polarities and functionalities has attracted researchers towards their use in various formats. Commercially available HLB particles are restricted in their size ranges, which are not suitable for some of the sampling devices when intended for the development of coating-based micro or nano-sized sampling devices. To address this, HLB particles with tunable physical characteristics were synthesized by using divinylbenzene and N-vinylpyrrolidone as monomer via precipitation polymerization. Factors affecting the synthesis conditions in precipitation polymerization like stirring speed, monomer amount, porogen, solvent for polymerization, amount of initiator, functional monomer and crosslinker ratio were optimized. Particles obtained after synthesis were characterized for their physical properties like size, surface area, porosity, pore volume and functional group information by SEM, TEM, surface area analysis, and FT-IR respectively. Particles in tunable sizes from 100 nm to 2 µm with meso or micropores were synthesized by varying porogen amount.

**Keywords:** Material Science, Particle Size and Distribution, Sample Preparation, SPME, Material Science, Particle Application Code: Material Science Methodology Code: Sampling and Sample Preparation

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Synthesis and Characterization of Hydrophilic-Lipophilic Balance Particles for Various Solid Phase Microextraction Sampling Devices

Hydrophilic-Lipophilic Balance particles (HLB) have emerged as a promising material during last two decades. Their ability to extract compounds of various polarities and functionalities has attracted researchers towards their use in various formats. Commercially available HLB particles are restricted in their size ranges, which are not suitable for some of the sampling devices when intended on the development of coating based micro or Nano sized sampling devices. To address this, HLB particles with tunable physical characteristics were synthesized by using divinylbenzene and N-vinylpyrrolidone as monomer via precipitation polymerization. Factors affecting the synthesis conditions in precipitation polymerization like stirring speed, monomer amount, porogen, solvent for polymerization, amount of initiator, functional monomer and crosslinker ratio were optimized. Particles obtained after synthesis were characterized for their physical properties like size, surface area, porosity, pore volume and functional group information by SEM, TEM, surface area analysis and FT-IR respectively. Particles in tunable sizes from 100 nm to 2 µm with meso or micropores were synthesized by varying porogen amount.

Keywords: Material Science, Particle Size and Distribution, Sample Preparation, SPME, Material Science, Particle
Application Code: Material Science
Methodology Code: Sampling and Sample Preparation
A Guide for HPLC Troubleshooting: How to Diagnose and Solve Chromatographic Problems?

High-performance liquid chromatography is one of the most used analytical techniques in industry. Regardless of whether the HPLC system is in well-maintained condition or not, it is impossible not to run into issues, such as: pressure ripple, artifact peaks, retention time variability, peak shape distortion, etc. Knowledge on how to diagnose, isolate, and fix a HPLC problem is essential for the success of analytical testing. This guide will be useful for analysts who are looking for a summary of the most common problems encountered in HPLC analysis along with troubleshooting clues on how to diagnose and solve these problems. Example cases from our labs and from the literature - are presented to support the decision tree demonstration.

Keywords: Chromatography, HPLC
Application Code: Other
Methodology Code: Liquid Chromatography
Assessing the Impact of Increased Pre-Column System Volume on Peak Shape for High Organic Diluent Samples Using U(H)PLC

Ideally when running a chromatographic method, sample diluent should be as close to method starting conditions as possible. This is done in order to minimise the possibility of band spreading and peak distortion which can lead to poor peak symmetry, peak splitting or unusable data. In practice this is not always possible as sample solubility often dictates the amount of organic content needed to ensure complete dissolution. With older, higher dispersion volume LC systems, this phenomenon is less problematic due to sufficient sample/solvent mixing which mitigated peak problems brought about by solvent effects. However for modern lower dispersion U(H)PLC systems, high organic diluents can be problematic when injected in larger volumes and can result in poor peak symmetry or splitting. To understand this phenomenon and investigate means for a simple solution to overcome this issue, five USP methods were selected (i.e. acetaminophen, itraconazole, ketoconazole, loratidine and bicalutamide) which require sample diluent organic levels ranging between 67- 100% organic.

Within this body of work, all methods were conducted on the Waters ACQUITY Arc system (system dispersion ~25µl) and the Waters ACQUITY UPLC H-Class (~7µl) with structured and iterative modifications to increase volume to assess the impact of additional volume on peak symmetry problems brought on as a result of high organic diluents.

Keywords: HPLC, Liquid Chromatography, Quality Control
Application Code: Quality/QA/QC
Methodology Code: Liquid Chromatography/Mass Spectrometry
These days, food safety is getting important to protect human health. In order to declare the food safety, test and confirmation by multiple analytical methods used in the food produce line have been spread. Shimadzu TOC analyzer make it possible to analyze TOC and TN easily and speedy and it would be good system to evaluate the quality of food. Additionally, the system can be an easy system to evaluate the cleaning of process line, “Cleaning Validation”. Cleaning validation has been getting important in food production field. Here we’d like to show the analysis method of nitrogen/carbon in seasoning and drink and the cleaning validation used in the food production line.

Keywords: Analysis, Food Contaminants, Food Safety, Total Organic Carbon
Application Code: Quality/QA/QC
Methodology Code: Atomic Spectroscopy/Elemental Analysis
Electrochemical battery testing was reviewed as it relates to hardware limitations, advanced techniques, and safety. Electrochemical battery testing is performed under either potentiostatic or galvanostatic control, and, in the case of packs with cells in series, results in the need to manage large potentials on the order of tens of volts or currents exceeding 1 ampere. Characterizing high-voltage packs often requires controlling potentials that exceed the compliance voltage of commercial potentiostat/galvanostats. This poster demonstrates how the potential range of a potentiostat/galvanostat may be extended to higher potentials using a voltage divider (or multiplier). Even with this precaution, battery testing can take the instrument or battery device to the limits of its specifications, whether it is from large potentials or currents, so the safety of the researcher and the instrument must be considered in the experiment design. The potential and current limitations of the instrument are related to its power management capabilities. Exceeding these specifications can lead to permanent damage of the equipment, or subtle drifts in the measurement that result in unreliable data, depending on the magnitude of the overload. In addition, working outside of the battery’s own specifications could lead to heating, expansion, and possible explosion of the specimen causing an unacceptable risk for the researcher. To this end, it is recommended to implement cutoffs on the maximum current, potential, or combined power associated with the electrochemical measurement. Implementation of these safety cutoffs are demonstrated on a commercial software platform for common techniques. Also, potentiostatic and galvanostatic intermittent titration techniques (PITT and GITT), techniques to determine diffusion coefficients of lithium ions in a solid state electrode material as a function of Li-ion intercalation loading, are presented in the context of these safety considerations.

Keywords: Electrochemistry
Application Code: Quality/QA/QC
Methodology Code: Electrochemistry
To ensure the reliability of amino acid analyses, the NMIJ has developed high-purity certified reference materials (CRMs) for 17 proteinogenic amino acids and 2 related compounds. We also performed calibration service for 22 amino acids analogs relevant to clinical chemistry, food chemistry, pharmaceutical biotechnology, and the accurate quantification of protein/peptides by amino acid analysis. These CRMs and RMs will be the primary standards used in the metrological traceability system. Thus, we ensured that the materials are of sufficient quality to be used as primary standards for the calibration of analytical instruments or reagents, and will improve the reliability of amino acid analyses. Previously, amino acid mixed standard solution was developed only by the National Institute of Standards and Technology (NIST) and a reagent company. The 17 proteinogenic amino acids were certified in this CRM. However, the number of amino acids actually analyzed in many laboratories is insufficient, with only 17 proteinogenic amino acids currently analyzed, so highly reliable standard solutions for other amino acids are necessary.

In this research, we established the preparation method and the uncertainty evaluation approach for accurate three kinds of mixed standard solutions of amino acids. The list of components in the mixed standard solutions of amino acids is shown to the Table. The accuracy of preparation was evaluated in a collaborative study with one other laboratory. In addition, the homogeneity and stability of the solutions were evaluated with pre-sample solutions.

Abstract Text

Co-Author(s) Ishikawa Junichi, Kurooka Kurooka, Nakayama Nakayama, Ozawa Shinichi, Tomoki Ito, Toshihide Ihara

Keywords: Amino Acids, Quality Control, Reference Material, Standards
Application Code: Quality/QA/QC
Methodology Code: Liquid Chromatography
Automated liquid handling (ALH) is an essential component of life science and chemical laboratories, however, the methods and practices for performing volume verifications have yet to be fully standardized and implemented. The International Organization for Standardization (ISO) has published generalized metrological standards for liquid handling, but the responsibility of applying these guidelines to the calibration of ALHs ultimately rests on individual labs and service companies that may lack the specific knowledge required. The lack of standard volume verification practices is particularly acute in high throughput, Genomics, Pharmaceutical, Chemical, and the like labs and when extremely low volumes (less than 1 microliter) are used. Our study addresses the fundamental metrological requirements of ISO certification and the application of these standards to ALH devices. We discuss physical requirements and how to create and maintain NIST traceability, as well as the training and certification requirements needed to ensure consistent application of metrological standards. Challenges unique to ALHs such as method development and optimization, software standardization, and high throughput volume verification will be addressed. The future development of ISO standards specifically targeting high throughput, low volume ALHs will be discussed in detail in the context of similar proposals such as IWA 15. We look at the requirements needed for training standards needed by test centers, training centers, instructors, auditors, service providers, vendors, and users.

Keywords: Laboratory Automation, Quality Control, Teaching/Education, Validation
Application Code: Quality/QA/QC
Methodology Code: Education/Teaching
Manufacturing of gluten-free products is usually characterized by the need of optimizing specific technological processes aimed at obtaining properties to fulfill consumer expectations, since the raw material used often gives poor results in terms of taste, aroma, consistency and nutritional properties. For these reasons, alternative ingredients are being tested with the aim of improving the final result.

Chestnut flour contains high quality proteins with a very good content of essential amino acids (4-7%), fibers (4-10%) and low fat (2-4%).

For optimization of a recipe with chestnut flour and peels to produce gluten-free biscuits, different samples were prepared by mixing chestnut flour/peels and gluten-free flour in different proportions. The behavior of the biscuits during 60 days of shelf-life has also been monitored. Increasing percentages of chestnut flour or peels lead to an improvement of color but too high percentages resulted in excessively hard texture. While the taste of the biscuits was improved with the addition of the chestnut flour, high percentages of peels were unpleasant to the sensory analysis conducted by ranking test. The formulations of biscuits with the addition of 50% chestnut flour and 5% peels resulted the most appreciated. Since oxidative stability is an important parameter affecting food shelf-life, especially for a fat-rich product such as biscuits, the product was submitted to analysis by Oxitest (VELP Scientifica, Italy), a reactor based on the use of high temperature and over-pressure of oxygen allowing to easily measure a sample oxidative stability by accelerating the oxidation process.

Obtained data showed promising results since the increase of percentage of chestnut flour and peels in the dough lead to a product with improved color, harder texture, better taste and enhancement of oxidative stability, opening new perspectives in the use of chestnut flour and peels for preparation of gluten-free products.

Keywords: Analysis, Food Science, Quality
Application Code: Food Science
Methodology Code: New Method
Stacking, or fusing, parallel regression results has been shown to reduce the error associated with multivariate calibration models and, therefore, the target properties they predict for experimental samples. Stacking makes use of all available data and weights the sections of the data that correlate strongly with the target property (e.g. analyte concentration) more than the sections that are less correlated. In this study, stacking is combined with partial least squares regression (referred to as stacked-PLS or SPLS) for the quantitative analysis of three different infrared spectral data sets. Stacking can be combined with other common multivariate regression methods as well, such as principal component regression (PCR). Other forms of multivariate data, other than infrared spectra, can also benefit from stacked analyses. We observe a significant decrease in the root mean square error of prediction (RMSEP) when utilizing SPLS compared to the results achieved from traditional PLS and interval PLS (iPLS), both of which are methods that do not involve stacking. The predictive ability of a calibration model depends on how well it is optimized. Thus, we present a unique two-way optimization that fully optimizes the two parameters that define a stacked calibration model, and, as a result, produces models that outperform those obtained using the previously reported optimization approach.

**Keywords:** Chemometrics, Data Analysis, Quantitative, Statistical Data Analysis

**Application Code:** Bioanalytical

**Methodology Code:** Chemometrics
Certified values and uncertainties are essential factors of certified reference materials. The procedure of uncertainty evaluation of reference materials is very complex due to applying plenty of statistical technology. We need to analyze the sources of uncertainties, draw cause-and-effect diagram, quantify the elements of uncertainty, combine the uncertainty values and obtain the compound uncertainties. The statistical approaches that we may use are normal distribution test, deletion of suspicious values, F test, t test, Cochran test, analysis of variance(ANOVA), linear regression. Because of the differences of chemical and physical property of materials, the methods of characterization vary greatly, therefore the uncertainties of characterization are more complicated.

Uncertainty evaluation technology group of chemical division of National Institute of Metrology(China) has developed an online information system for uncertainty evaluation of reference materials, which meets the requirements of ISO Guide 35:2015 and ISO/IEC 98-3:2008 Guide to the Expression of Uncertainty. The system consists of four major functions: formula compiling engine, template editing and quoting, data storage and treatment, user’s roles and authorities.

Formula compiling engine is the core of the system, by which users can edit the math formulas and the system can compile the formulas. By the function of templates management, researcher of reference materials can define the evaluation procedure, set the calculation formula for every uncertainty factor and publish the template. The producer of reference materials can quote a standard template, input and store the data. The system can treat the data immediately, display the results and give the cause-and-effect diagram to help understanding. By the roles and authorities management function, administrator can manipulate the system to ensure the safety, sustainability and stability of running. The system is currently in internal testing.
Abstract Text
Multivariate calibration models are essential in science as they allow scientists to predict unknown properties of experimental samples. The quality of a calibration model is related to the amount of uncertainty associated with the properties it predicts. Stacked Moving Window Partial Least Squares Regression (SMWPLS) has recently been proposed as a technique to develop calibration models capable of emphasizing and utilizing only the most informational and relevant sections of the calibration data to better predict properties of interest (e.g. analyte concentration). Consequently, these models can achieve lower levels of uncertainty compared to calibration techniques currently used today.

In this study, SMWPLS is being applied to infrared spectra of microalgae samples grown in various, controlled chemical environments. By analyzing these spectra with SMWPLS, it is possible to predict the chemical profile in which these microalgae grew. Specifically, we are modeling the concentrations of nitrate, carbon dioxide, and ammonium within the immediate growing environment. SMWPLS is shown to decrease the uncertainty associated with the predicted concentrations by over 50% when compared to other commonly used calibration models. The predictive ability offered by SMWPLS could, in the future, aid in the use of microalgae as in situ sensors for water quality monitoring. As with many multivariate models, SMWPLS must be optimized in order to achieve peak predictive performance. SMWPLS requires a unique three-way optimization that yields the best combination of the three parameters that define this method. This optimization can often impose a substantial strain on the available computational resources. Therefore, we provide an effective optimization approach that first involves a lower resolution scanning of the different combinations of these parameters to produce an acceptable combination. Next, a localized high-resolution scan is performed to further improve this combination.

Keywords: Chemometrics, Data Analysis, Quantitative, Statistical Data Analysis
Application Code: Bioanalytical
Methodology Code: Chemometrics
Precise and accurate titration results can be achieved with a standardized and constant titration process. One important step in the titration procedure is the correct transfer of data from the reagent and standard to the instrument software. Chemical name, concentration, batch number, shelf life, article number, traceability are parameters which are important for the documentation in accordance with quality assurance guidelines. This information is also essential for the calculation of the results. A new generation of titrators with an RFID reader together with reagents & standards equipped with an RFID chip will transfer the data automatically. This avoids data transfer failures and makes the titration process faster and easier. All data will be saved and stored in the instrument software or on the chip and can be used for the calculation of results or gives warning when the shelf life will be expired, additionally data for regulatory requirements are also saved. In GMP rules, the fundamentals of data integrity are described by the acronym ALCOA. According to these rules, data have to be Attributable, Legible, Contemporaneous, Original and Accurate. Particularly when combined with the PC software, these new types of reagents represent a significant milestone in fulfilling the ALCOA rules, offering an innovative data integrity solution for information transfer from titrant and standard to titrator. Data stored in the titrator are thus complimented with source information and full data traceability, and an electronic audit trail is ensured. After transferring all data, the titration process can start. A correct standardization with a titrimetric standard is important for reproducible titration results. The instrument will calculate with the correct data delivered through the RFID chip from the standard like molecular weight or assay. Calculated factor from the standardization is now used for the correct calculation of the results from the samples.

Keywords:  Standards, Titration
Application Code:  Other
Methodology Code:  New Method
Sample Preparation and Chromatography

Transition Metal Oxide-based Sol-Gel Sorbents with Electrically Charged Ligands for Enhanced Enrichment of Phosphopeptides, Nucleotides, and Related Biomolecules by Capillary Microextraction Online Coupled to HPLC

A major factor determining the extraction ability of a sorbent is the diversity of molecular level interactions it can undergo with the target analyte molecules. Such molecular level interactions must be strong enough to overcome those between the target analyte and the sample matrix. Therefore, a key consideration in the design of an efficient sorbent is envisioning a material structure that maximizes the variety of molecular level interactions that the resulting sorbent is expected to offer. Besides, effective enrichment of important biomolecules including those in the title often require highly acidic or basic extraction conditions. Unlike traditional silica-based extraction media that fail to ensure sorbent stability under such conditions, transition metal oxides possess exceptional pH stability. They also have Lewis acid sites capable of providing Lewis acid-base interactions with analytes containing Lewis base sites (e.g., phosphopeptides and nucleotides). These attributes of transition metal oxides can be effectively utilized in the enrichments of trace/ultra-trace concentrations of the aforementioned biomolecules. Covalent anchoring of an electrically charged organic ligand to the sorbents would further enhance their extraction ability by additionally providing electrostatic- and van der Waals interactions. To this end, we utilized the principles of sol-gel chemistry to create tantalum- [J. Chromatogr. A, online published Sep 22, 2017], niobium- [J. Sep. Sci. 2017, submitted], and zirconium-based [J. Chromatogr. A 2016, 1468, 23-32] organic-inorganic hybrid sorbents. A comparison of their performances in capillary microextraction (CME) [Anal. Chem. 2002, 74, 752-761] with those of the state-of-the-art titania-based sorbents of analogous structures revealed that the new transition metal oxide-based sol-gel sorbents outperformed their titania-based counterparts by providing up to 50% higher extraction efficiency and superior desorption efficiency (96% vs. 90%).

Keywords: Extraction, HPLC, Nucleic Acids, Peptides
Application Code: Bioanalytical
Methodology Code: Sampling and Sample Preparation
At present within the United States, there are 8 states with legalized recreational marijuana usage and 21 states with medical marijuana legalized. As this trend continues, consumer safety is a major topic of concern. One area of considerable concern is the screening for pesticides. Like most agricultural products, pesticides are widely used for crop management and can find their way into consumer goods. It is therefore important that reliable, rapid and cost effective procedures be in place for the screening of products destined for a consumer market.

Pesticides extractions and analysis have long been in place for the food and environmental industries. Tapping into these methodologies, the usage of Pressurized Liquid Extraction (PLE) can be fitted to deliver a one step extraction and extract clean-up process for rapid LC/MS-MS analysis of a wide array of pesticides.

Extraction cells were filled with inert material, sample aliquots, clean-up sorbents and spiked with relevant standards. Cells were sealed and loaded onto the PLE extraction system. Cells were filled sequentially with acetonitrile, pressurized and held at ambient temperature for 5 minutes. The cells were then depressurized and flushed with nitrogen to purge remaining solvent into the collection tubes. From the final extract an aliquot was transferred to a vial for LC/MS analysis.

Performance of in-cell clean-up using traditional SPE sorbents proved to be highly efficient at removing non-target interferences. The process of performing the clean-up in-cell required no additional sample prep steps to be employed thus enabling a true one step automated extraction. Analysis of the extracts (spiked at 0.1 ug/g) showed very good recoveries of an extensive list of pesticides ranging across multiple chemical classes. Reproducibility of the extract sets yielded good RSDs for most analytes.

Pressurized Liquid Extraction of marijuana products yielded fast and reliable data on pesticides contamination.

Keywords: Drugs, Gas Chromatography/Mass Spectrometry, Pesticides
Application Code: Safety
Methodology Code: Sampling and Sample Preparation
A novel analytical approach was utilized for identification of compounds in two standard reference materials (SRM, smoker’s and non-smoker’s urine). These reference materials were vital in the diagnosis and setup of standard operating procedures for metabolic profiling of urine in humans. The applied methodology included a combination of comprehensive chromatography coupled to high performance time of flight mass spectrometry (GCxGC-TOFMS). Characterization of samples was conducted using both targeted and untargeted processing methods. Analyses of the standards resulted in composition maps (Contour plots) displaying wide variety of functionality (e.g., polyaromatic hydrocarbons, phthalate metabolites, phenols and pain killers) and compound derivatives. These plots were highly structured showing clustered classes of compounds and provided high quality spectral data that were searched against large, well-established databases. These full mass range, historical data archives were probed collectively to identify important classes of compounds and clearly distinguish the sample types.
Marijuana has been legalized in several states. Cannabis has a number of components that are classified as cannabinoids. Three cannabinoids of main interest are tetrahydrocannabinol (THC), cannabidiol (CBD) and cannabinol (CBN). The work presented here focused on potency identification and quantification of the THC:CBD ratio which is the main objective in the analysis of cannabis. Medical marijuana has higher levels of CBD and lower levels of THC. The therapeutic CBD is desirable for medicinal effect; the psychoactive THC is sometimes undesirable. The THC: CBD ratio is important to medical professionals prescribing cannabis for medicinal purposes. Edibles, extracted liquids, and solids must be tested also for potency. This application focuses on the potency of the flower portion of the plant.

Automated Pressurized Liquid Extraction was used to extract 1-5 g of sample which had been mixed with inert Hydro-matrix®. The sample was place in the extraction cell that was then capped with disposable Teflon end caps. It was extracted for 1 min at ambient temperature and 1500 psi in 100% methanol. A nitrogen flush transferred the analytes to 250 mLs evaporator collection tubes. Samples were analyzed with LC/MS.

Analysis of a reference mix of tetrahydrocannabinol, cannabidiol, and cannabinol (all at 100 µg/mL) all showed excellent extraction efficiency. Similar results were obtained for the processing and analysis of 2g and 5g of flower.

Rapid and reliable automated extraction of cannabis is a great asset in the expanding laboratory testing of marijuana.
First and second generation anticoagulant rodenticides, which act as vitamin K enzyme inhibitors, are widely used for rodent control. Exposure to these rodenticides show a decrease in blood clotting factors and result in excessive internal and external bleeding in the exposed organism. While these compounds are effective in the control of rodent populations, the potential exists for exposure of non-target species through bait ingestion, scavenging of poisoned target species or intentional poisoning. The analysis of hydroxycoumarine (bromadiolone, brodifacoum, coumachlor, coumatetralyl, difenacoum, flocoumafen and warfarin) and indandione (diphacinone and chlorophacinone) derivatives typically involve extraction of liver, blood, urine or other suitable tissues matrix; solid phase extraction (SPE) cleanup to remove co-extracted interferences; and quantification by high performance liquid chromatography (HPLC).

In this study, we analyze three of the most common matrices (liver, blood and urine) used to determine poisoning in non-target species. The fortified matrices were extracted with suitable techniques to recover the hydroxycoumarine and indandione derivatives and SPE cleanup techniques were applied to determine the most suitable stationary phase to remove co-extracted interferences and maximize recoveries of the analytes of interest. The labor-intensive steps of SPE conditioning, loading and elution were reduced to two steps: 1) loading sample extracts on to the instrument and 2) programming the sequence with saved method parameters. This provided a fast and simple automated method for the labor intensive process of manually conditioning the SPEs, loading the extracts on to the substrate, removal of impurities and recovery of the analytes of interest.

Keywords: Bioanalytical, Biological Samples, Sample Preparation, Solid Phase Extraction
Application Code: Bioanalytical
Methodology Code: Sampling and Sample Preparation
The demand for water in unconventional oil and gas reservoirs is steadily increasing. This has driven the development and implementation of novel treatment processes of drilling waste fluids for reuse or recycling purposes. These treatment approaches typically reduce the risk of these fluids negatively effecting the environment through transportation, spills during handling, and waste injection wells. The high ionic strength and varying organic nature of this waste stream makes treatment and analytical analysis difficult. Two sample preparation techniques, liquid-liquid extraction according to EPA 3510C and solid phase microextraction with SPME Arrow (Restek Corp., Bellefonte, PA), will be compared while analyzing finished treated waters of the Eagleford Shale and Permian Basin of Texas. Emulsions are a common difficulty encountered during the traditional liquid-liquid extractions of these samples. Multiple SPME fibers will be scouted to identify the most appropriate for efficient extraction in such high ionic strength solutions. SPME offers significantly less sample consumption (20 mL vs 1000 mL), extraction without nearly 500 mL of methylene chloride, and shorter preparation time. Chromatograms of each sample are of greater quality when using SPME due to the elimination of injection solvent and impurities found within. Advice will be given towards other analytical advantages, predominantly related to extraction selectivity and sensitivity between the two protocols. Accurate measurements of the treatment finished product are crucial towards optimizing treatment processes and meeting standards for discharge or energy operator reuse.

Keywords: Environmental/Waste/Sludge, Petroleum, SPME, Water
Application Code: Environmental
Methodology Code: Sampling and Sample Preparation
Ligand Exchange Supercritical Fluid Chromatography: Separation of Stereoisomers of 1,2,4-Trivinylcyclohexane

The stereoisomeric structure of a chemical intermediate can play a large role in the activity of the final synthetic molecule. One such material, 1,2,4-trivinylcyclohexane (TVCH), is used as an intermediate in chemical synthesis routes. Given that it has four stereoisomers, it was hypothesized that these could play a large role in the properties of the final synthetic material. Therefore, it was desired to separate the stereoisomers and purify the TVCH compound to test this hypothesis.

Gas chromatography and liquid chromatography (LC) have been used to separate stereoisomers. These were evaluated for TVCH separation, but only 3 of the 4 stereoisomers could be resolved. Therefore, supercritical fluid chromatography (SFC) in the ligand exchange mode was investigated. Ligand exchange chromatography based on silver-loaded silica columns and liquid mobile phases has been used in the past to separate molecules containing vinyl groups. SFC has been shown to offer increased retention and rapid analyses as compared to LC. Using SFC in the ligand exchange mode, all four stereoisomers of TVCH were separated. Short analyses times were observed which suggests that this technique may be used in a preparative mode for isolation of synthetically relevant amounts of material.

Acknowledgement
The authors gratefully acknowledge the funding support of The Dow Chemical Company for this work.

Keywords: Chromatography, Prep Chromatography, Supercritical Fluid Chromatography
Application Code: Other
Methodology Code: Supercritical Fluid Chromatography
Sample Preparation and Chromatography

Determination of Aflatoxins in Corn Products Using Dispersive Pipette Extraction (DPX) Followed By High Performance Liquid Chromatography (HPLC) Analysis

Aflatoxins are cancer-causing toxins that are produced by the molds Apsergillus flavus and Aspergillus parasiticus. Aflatoxins can contaminate a wide range of food products such as corn, peanuts, pistachios and cereal based foods. Traditional methods for extraction and detection of aflatoxins is based on immune-affinity column for sample cleanup followed by liquid chromatography (LC) or capillary electrophoresis (CE) analysis. These sample preparation methods, however, are expensive, require many steps, and increase analysis time. This study presents a rapid and simple sample preparation method for the determination of Aflatoxins in corn products using Dispersive Pipette Extraction (DPX), the average time consumption for extracting of one sample is less than 5 minutes. High Performance Liquid Chromatography (HPLC)/UV-vis was used to analyze aflatoxins. Different solid phase extraction sorbents and solvents were evaluated, and the SDVB (styrene divinylbenzene) sorbent with acetonitrile elution were found to provide the highest recovery. The chromatographic analysis of aflatoxins was accomplished using a C18 column and UV-vis detection. Recoveries were above 90% for aflatoxins in the corn sample spiked from 2-100 ppb and average relative standard deviation was below 10%, which indicates good accuracy and precision of the developed method.

Keywords: Separation Sciences, Solid Phase Extraction
Application Code: Food Safety
Methodology Code: Sampling and Sample Preparation

Abstract Text

Aflatoxins are cancer-causing toxins that are produced by the molds Apsergillus flavus and Aspergillus parasiticus. Aflatoxins can contaminate a wide range of food products such as corn, peanuts, pistachios and cereal based foods. Traditional methods for extraction and detection of aflatoxins is based on immune-affinity column for sample cleanup followed by liquid chromatography (LC) or capillary electrophoresis (CE) analysis. These sample preparation methods, however, are expensive, require many steps, and increase analysis time. This study presents a rapid and simple sample preparation method for the determination of Aflatoxins in corn products using Dispersive Pipette Extraction (DPX), the average time consumption for extracting of one sample is less than 5 minutes. High Performance Liquid Chromatography (HPLC)/UV-vis was used to analyze aflatoxins. Different solid phase extraction sorbents and solvents were evaluated, and the SDVB (styrene divinylbenzene) sorbent with acetonitrile elution were found to provide the highest recovery. The chromatographic analysis of aflatoxins was accomplished using a C18 column and UV-vis detection. Recoveries were above 90% for aflatoxins in the corn sample spiked from 2-100 ppb and average relative standard deviation was below 10%, which indicates good accuracy and precision of the developed method.

Keywords: Separation Sciences, Solid Phase Extraction
Application Code: Food Safety
Methodology Code: Sampling and Sample Preparation
Sample Preparation and Microextraction

Development of a Biocompatible In-tube Solid Phase Microextraction Device: A Rapid and Sensitive Approach for Direct Analysis of Single Drops of Complex Matrices

Current advancements in analytical instrumentation as well as sample preparation techniques enabled the possibility of sample preparation devices miniaturization in cases where only a very limited sample volume is available and when rapid analysis or high throughput are required, without the risk of sacrificing sensitivity. The presented study aims to develop a sensitive solid phase microextraction (SPME) based device for direct and rapid analysis of untreated complex matrices (i.e. 2 µL of blood) using a thin layer of biocompatible nano-structured polypyrrole (PPy) as an extracting phase, electrochemically deposited inside of a spinal needle in the presence of a surfactant providing improved coating characteristics. Two strategies were employed for analysis of the target compounds, including offline desorption followed by HPLC-MS/MS analysis as well as direct to MS coupling. The total analysis time per sample was effectively decreased by the short time needed to reach the extraction equilibrium (i.e. t•2 min). The in-tube SPME device provided satisfactory sensitivity for a number of compounds demonstrating wide range of physicochemical properties, with high total recoveries due to the large volume and capacity of the coating. Investigation of matrix effect for the prepared device in whole blood samples resulted in encouraging results in the range of 83-120 %, attributed to the smooth surface and small pore size of the biocompatible PPy coating. The demonstrated rapid analysis of whole blood samples due to elimination of time-consuming sample pre-treatment steps deems the developed probe and sampling protocol suitable for application in the field of forensic toxicology as well as drug monitoring for clinical purposes. Moreover, the simplicity of the developed probe translates to its versatility, enabling its use in a multiple configurations depending on the analysis’ objective and available analytical instrumentation.

Keywords: Clinical/Toxicology, Liquid Chromatography/Mass Spectroscopy, Sample Preparation, SPME
Application Code: Clinical/Toxicology
Methodology Code: Sampling and Sample Preparation
Miniaturisation of solid phase extraction (SPE) techniques provide enormous potential for performing sample preparation on small sample sizes such as biological and environmental fluids. Automation of micro solid-phase extraction (µSPE) offers high reproducibility with small solvent and sample volumes in the µL range.

[µSPE]ed cartridges employ sorbent particle sizes of 3 [µ]m or less which provides a high surface area for efficient extractions and greater selectivity and separation of target analytes compared against conventional SPE.

The most common SPE sorbents are organic bonded silica, such as C18, often used for sample clean-up or pre-concentration. By customising generic solid supports with functional groups, a variety of ligands may be bound to for targeted solid phase extraction. Such ligands may include antibodies and enzymes.

This poster will detail novel customised packings from generic silica materials in automatable µSPEed cartridges, and the conjugation of trypsin using cross-linking chemistry for enzymatic digestion of protein samples.

**Keywords:** Bioanalytical, Modified Silica, Protein, Sample Handling/Automation

**Application Code:** Bioanalytical

**Methodology Code:** Sampling and Sample Preparation
Online Solid Phase Extraction of Novel Psychoactive Substances from Biological Matrices

This research is exploring the use of online solid phase extraction (SPE) to extract novel psychoactive substances (NPS) of various drug classes from urine and whole blood. This method can increase throughput, decrease sample preparation time, and decrease sample handling. Some commonly used extraction/purification techniques are solid phase extraction (SPE), liquid-liquid extraction (LLE) and dilute/crash and shoot. These techniques can be expensive, time consuming, and may not eliminate all matrix effects. Although extraction techniques for common drugs of abuse are well studied, development of extraction methods specifically targeting NPS is needed due to the high prevalence of NPS in forensic casework.

Previous work on a validated screening/confirmatory tMRM method for 826 NPS by LC-QqQ-MS is being used to analyze all extracts for this study. The method allows for the screening of a wide variety of NPS drug classes and metabolites, with a focus on synthetic stimulants and cannabinoids. Online SPE is being evaluated using a mix of 29 NPS, which includes synthetic cannabinoids, synthetic stimulants, and metabolites in addition to other NPS. Evaluation is based on drug recovery, drug retention, reproducibility, minimization of matrix effects, time, and overall cost.

An Agilent 1290 Infinity HPLC system and Agilent 6460 QqQ-MS with Jet Stream Technology ESI was used for this research with an Agilent Zorbax Rapid Resolution HD Eclipse Plus C18 column for chromatographic separation. Online SPE was performed using an Agilent 1290 Flex Cube LC unit and a Bond Elute (BE) online polymeric sorbent material (PLRP-S) cartridge. Online SPE incorporates sample preparation as part of the analysis of samples rather than being a separate step, potentially increasing throughput. This research will lead to the optimization of an extraction technique that is capable of handling high throughput and NPS of many different classes.

Funded by NIJ Award # 2014-R2-CX-K006

Keywords: Forensics, On-line, Solid Phase Extraction, Toxicology
Application Code: Clinical/Toxicology
Methodology Code: Sampling and Sample Preparation
Silver nanoparticles (AgNPs) have many beneficial properties which render them useful for a variety of applications; such as catalytic and antibacterial agents among others. Thus, there is a continuing need for facile methods for the synthesis of uniform sized/shaped AgNPs in concentrated form which are stable during storage. Previously, we reported the one-pot synthesis of monodispersed highly concentrated gold nanoparticles via use of thermoresponsive zwitterionic N,N-dimethyl-alkyl-ammonio-propyl sulfate (C[n]APSO[4]) surfactants in conjunction with a cloud point extractive (CPE) step.\(^1\) In this presentation, we report on the one-pot synthesis of AgNPs under mild conditions using such zwitterionic surfactants and CPE. The effect of experimental conditions upon the synthetic procedure was evaluated and the AgNPs characterized by UV-visible absorption, dynamic light scattering and transmission electron microscopy measurements. The AgNPs were produced via reaction of silver ion with reductant at elevated temperature in the homogeneous solution followed by cooling and CPE such that the AgNP appeared in concentrated form in the surfactant-rich phase. The process required 8 min yielding stable 2.5 nm (n=50) sized AgNPs with synthetic product and extraction yields of 99.9 and 98.6%, respectively. The AgNP concentration (in the surfactant rich phase) was 4.75\(\times\)10\(^{12}\) particle/mL which is ca. 20 times greater than that of most commercially available products.

### Sample Preparation and Microextraction

**A New Titration Platform for Faster, Safer and Easier Analysis**

Deadlines, high sample throughput, increasingly strict standards in safety and quality – this is life in the laboratory. OMNIS is the new titration platform from Metrohm that makes titration safer, faster and easier than ever. By intuitive automation of 4 simultaneous analysis, sample throughput is increased by 60% and new, contact-free reagent exchange makes changing reagents safe and hassle free. The first ever, truly modular titration platform is designed to grow with the needs of your laboratory. OMNIS can be configured to perform today’s tasks. When future demands occur, OMNIS can be expanded. OMNIS is titration on a whole new level.

**Keywords:** Laboratory Automation, Sample Handling/Automation, Titration

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

**Methodology Code:** Sampling and Sample Preparation
Sample preparation step demands considerable efforts to provide suitable sample matrix and analyte conversion for chemical analysis. Thus, new procedures that allow reduced time and low reagent consumption are desirable. In this work, a new approach on sample preparation was proposed using vessel-inside-vessel strategy for the generation of auxiliary reagent in gas phase by the thermal decomposition of high concentrated ammonium persulfate solution. The proposed decomposition process was evaluated for whole milk sample. All assays were carried out a microwave oven (Start-D, Milestone) with closed vessel containing 8.0 mL of diluted nitric acid (1.0 and 2.0 mol/L) and an auxiliary digestion borosilicate tube filled with 3.5 mL of $(\text{NH}_4)_2\text{S}_2\text{O}_8$ (3.0 mol/L) in $\text{H}_2\text{SO}_4$ 0.9 mol/L and closed with Teflon membrane to permit gas diffusion without expel solution. Heating programs evaluation were performed by time (30 or 43 min) and temperature (180 to 210 °C) based on digests visual aspects, dissolved organic carbon content (DOC), recoveries obtained for four certified reference material (CRM’s) by ICP OES. Dissolved organic carbon concentration was about 20% for all digested obtained regardless of presence of auxiliary digestion tube contain persulfate. The digests generated employing the proposed strategy were colorless and presented reduced staining, especially for CRM’s with high fat content. The proposed method was applied to CRM’s with different fat contents, recoveries from 87 to 104% were obtained, which indicates good accuracy of the method except for Si, Cu, Cr, Fe and Pb, due to desorption and acid lixiviation from borosilicate tube walls. Finally, the proposed method allowed the determination of Ca, Cu, Fe, K, Mg, Na, P and Zn in whole milk powder samples, showing clear appearance in digests obtained for less drastic decomposition.

**Keywords:** Elemental Analysis, Method Development, Microwave, Sample Preparation

**Application Code:** Process Analytical Chemistry

**Methodology Code:** Sampling and Sample Preparation
Sample Preparation and Microextraction

The Combination of 3 New Sampling Techniques paired with GCMS for Determination of Uptake Rates and Accurate Monitoring of SVOC Endocrine Disruptors in Indoor Air

Growing understanding of endocrine disruptors continues to drive the need for accurate exposure assessment. Current monitoring techniques include active and passive air sampling. Passive samplers are more economical than active sampling systems requiring pumps in the field, however, currently there is no accurate way to determine SVOC passive uptakes rates. The combination of 3 new approaches provides a solvent-free and cost-effective manner of monitoring organic contaminants in indoor air by providing a way to collect compounds, measure diffusive uptake rates to convert the amount collected to concentrations present in air, and calibrate the GCMS to convert response to amount on tube by analyte weight. Reproducible passive sampling is performed using a diffusive sorbent tube without pumps or power source. Determination of diffusive sampling rates is achieved by using a 2nd similar device which actively collects and measures the volume collected, while maintaining near diffusive flow rates in adsorption to ensure identical recoveries. Active air sampling at flow rates slow enough to be comparable to diffusive rates allows the first-ever accurate comparison of active to passive collection, resulting in true determination of SVOC uptake rates. GCMS calibration response for all compounds collected in indoor air sampled is attained using a 3rd technique which allows liquid standards to be injected into a vial with subsequent vacuum assisted transfer via diffusion to the sorbent tube, creating the same distribution of the calibration compounds as obtained using passive samplers in the field. Passive samplers are placed in an environment for a period of hours to weeks, depending on the sensitivity and monitoring period required, and then isolated and transferred to a laboratory for direct thermal desorption onto a GCMS. Data reveals both the accuracy and reproducibility achieved by combining these 3 new approaches resulting in comprehensive SVOC air monitoring.

Environmental/Air, Sample Introduction, Sampling, Semi-Volatiles

Environmental

Sampling and Sample Preparation
Sample Preparation and Microextraction

A New Energized Dispersive Extraction Method for the Extraction of Semi-Volatile Organic Compounds from Soil

There is an ever-growing presence of organic compounds in the environment produced from both human activity and natural processes. Many of these organic compounds can be harmful to our health and are listed by the Environmental Protection Agency as priority pollutants. A number of these pollutants exist in a subset known as semi-volatile organic compounds. There is an increasing need to regulate and monitor these semi-volatile organic compounds in soil. Traditional methods to extract these compounds from soil, such as Soxhlet, can be time consuming and tedious. Presented here is a new energized dispersive extraction method in which 30 g of soil is extracted in 5 min; this includes cooling, filtering, and washing. This method is simple, fast and effective for many environmental extractions, including the difficult extraction of semi-volatile organic compounds.

Keywords: Environmental/Soils, Extraction
Application Code: Environmental
Methodology Code: Sampling and Sample Preparation
Sample Preparation and Microextraction

Automated Dispersive Liquid-liquid Microextraction Based on Solidification of Floating Organic Drop

In this work, 3D printing, open source robotics and flow based techniques have been combined synergistically to explore new boundaries in automated analytical sample preparation. As a result, the dispersive liquid-liquid microextraction technique based on the solidification of the organic phase (DLLME-SFO) has been automated for the first time. Automated in-syringe DLLME is carried out in a sequential injection system. After DLLME, phase separation is carried out in a 3D printed device mounted on a Peltier cell. In this device the organic phase is solidified facilitating phase separation, followed by the melting and collection of the extract. The phase separator is mounted on a multi-axis robotic arm and can be displayed in the optimum positions for phase separation, or to collect the obtained extracts in an autosampler. The control of the Peltier cell and the robotic arm are programmed by Arduino, and their action is integrated with the flow-based instrumentation in a single software package (AutoAnalysis). The resulting method showed a linear range for the determination of parabens by liquid chromatography from 2 to 50 \( \mu \text{g L}^{-1} \), with a LOD and LOQ of 2 and 3 \( \mu \text{g L}^{-1} \), respectively (4 mL of sample and 1 mL of organic phase made of dodecanol:MeOH, 15:85, v:v). The use of the selected extractant/disperser showed good compatibility with the polymer material of the 3D printed device. The developed method was applied as proof of concept to the determination of parabens in different types of water, urine, saliva, and personal care products.

**Abstract Text**

**Primary Author**
Alvaro J. Santos-Neto
University of São Paulo

**Co-Author(s)**
Deyber A. Vargas-Medina, Fernando Maya, Victor Cerda

**Keywords:** Automation, Extraction, HPLC, Sample Preparation

**Application Code:** General Interest

**Methodology Code:** Sampling and Sample Preparation
Alcoholic and non-alcoholic beverages are enjoyed by millions of people around the globe. The aroma and flavor profiles of each product are unique and made up from a variety of semi-volatile and volatile compounds including aldehydes, ketones, acids, alcohols, terpenes, esters and other trace level compounds. Monitoring of these compounds is necessary for the manufacturers to ensure consistent product quality. Analysis may also be necessary due to off flavor or odor complaints. The wide range of concentration, polarity and functional groups composing a flavor/aroma profile can make the analysis of the sample difficult. Techniques which are simple, use little or no solvent and encompass a wide range of analytes are desirable.

Thin Film Solid Phase Microextraction (TF-SPME) is an extension of regular SPME. TF-SPME is more sensitive than regular SPME due to the increased surface area and phase volume. The TF-SPME device is a 20 mm x 4 mm carbon mesh sheet which is impregnated with a sorptive phase. The TF-SPME devices can be used in headspace or immersive modes. In headspace mode, the TF-SPME device is suspended above a solid or liquid sample in an enclosed vial. In immersive mode, it is placed directly in a liquid sample. In both cases, the sample is agitated by stirring. After extraction, the devices are blotted dry and placed in an empty thermal desorption tube. They are analyzed by thermal desorption GC/MS. This technique is simple to use and requires no solvent.

This work will show the application of TF-SPME to the analysis of aroma and flavor components in alcoholic and non-alcoholic beverages.

Keywords: Beverage, Gas Chromatography/Mass Spectrometry, Sample Preparation, Thermal Desorption
Application Code: Consumer Products
Methodology Code: Sampling and Sample Preparation
Session Title: Sample Preparation and Microextraction

Abstract Title: Optimized IC Analysis of Anions and Organic Acids in Wine

Primary Author: Ashley Wittig
Author: Metrohm

Co-Author(s): Tom Zarella

Abstract Text
Product consistency and quality is of utmost importance to winemakers. It is also critical to monitor and evaluate yeast performance and efficiency throughout the fermentation process. Wine analysis can aid vintners with ensuring predictable flavor and aroma characteristics in finished product by monitoring common indicators of acidity, mouthfeel, and balance. It also evaluates nutrients and other additives which could potentially have deleterious effects on efficiency and production during the fermentation process. This work shows the use of ion chromatography to analyze red and white wine for chloride, phosphate, sulfite, sulfate, malate, tartrate, and oxalate. Sequential suppression and conductivity detection was used in combination with automated, in-line filtration. This rapid, isocratic analysis enables high throughput laboratories to maximize production while maintaining simple instrument operation.

Keywords: Analysis, Food Science, Ion Chromatography
Application Code: Food Science
Methodology Code: Sampling and Sample Preparation
Abstract Text

Raman spectroscopy is a powerful tool that provides real-time in situ measurements that inform on chemistry from laboratory discoveries to mega-scale manufacturing. Representative sampling of a chemical reaction or process stream is an important aspect of ensuring a robust method, as incomplete sampling or sub-sampling may not adequately capture variations in the process. Solids and turbid media exhibit optical scattering and absorption, both of which affect the sampling volume of an optical probe and recovery of Raman-scattered photons. New approaches in sampling for Raman spectroscopy in solids or turbid media provide representative sampling and enable in-process corrections. We discuss variants of Raman spectroscopy where the geometry of the optical fibers with respect to the sample can be varied in order to optimize signal collection. Backscattered Raman, wide-area (or large volumetric) Raman, and enhanced reflection Raman are compatible with in-process measurements. Transmission Raman is a powerful off-line tool for measuring chemical heterogeneity within a solid or turbid sample. We discuss practical and theoretical considerations of optical scattering and sampling geometry in polymer and life science applications. In one example, a hybrid sampling approach used both backscattered Raman and large volumetric Raman, provide multi-scale measurements in-process, in order to improve understanding of a polymerization reaction. Examples in pharmaceutical and bioprocessing will illustrate Raman’s capability for providing robust measurements in media exhibiting optical scattering.

Keywords: Biopharmaceutical, Pharmaceutical, Polymers & Plastics, Raman Spectroscopy
Application Code: Process Analytical Chemistry
Methodology Code: Vibrational Spectroscopy
Moist snuff is the most popular oral tobacco product in the United States, accounting for 87% of the 2013 smokeless tobacco market. Moist snuff tobacco is sold in a variety of cut sizes, flavors, and styles, all of which influence the products’ addictiveness, attractiveness, and toxicity. We tested the levels of total nicotine, pH, unprotonated (free) nicotine, minor alkaloids, flavors, and tobacco-specific N’-nitrosamines (TSNAs) in forty oral tobacco products from four leading moist snuff brands. These tobacco selections were chosen for analysis based on their popularity, novelty (e.g., ready cut, x-tra tobacco), or history of unreported changes to product formulation. The concentration of total nicotine ranged from 9.74 to 15.8 mg/g and pH ranged from slightly acidic to slightly basic (pH 6.56 to 8.33). Based on the measured product pH, the amount of free nicotine spanned from 0.37 to 6.58 mg/g, illustrating a 17-fold variation. The most abundant flavor compounds, menthol, methyl salicylate, and ethyl salicylate were detected equally in 47.5% of the brands, and anatabine (170 – 447 μg/g) and nornicotine (136 – 245 μg/g) contained the highest minor alkaloid levels. Our research reflects the diversity of chemical constituents and product styles within the rapidly growing moist snuff industry. Monitoring of moist snuff products is necessary as smokeless tobacco use continues to expand, novel brands are introduced, and classic products are rebranded for current market use. These data may help inform public health and regulatory officials on harmful or potentially harmful constituents levels between smokeless products types and brand specific changes over time.

Keywords: Characterization, Chromatography, GC-MS, Liquid Chromatography/Mass Spectroscopy
Application Code: Process Analytical Chemistry
Methodology Code: Gas Chromatography/Mass Spectrometry
Determination and Quantification of Ethyl Xanthate (Pex) by UV-Vis Spectroscopy

This work develops an analytical methodology for determination and quantification of PEX collector in water samples. The collector was characterized using UV-Vis spectroscopy. A sweep between 210 – 350 nm was performed in a 10 mg L-1 PEX solution at pH 10 using 0.1 mol L-1 ammonia buffer solution. The absorbance peaks were obtained at 226 nm and 301 nm (Fig. 1).

The PEX collector presented a linear range between 2-20 mg L-1 with a linear regression of $\text{y} = 0.1067 + 0.0077$ and Pearson regression (R2) of 0.9998, observing a linear behavior in the analyzed PEX concentrations (Fig. 2). The limit of detection (LOD) and quantification (LOC) calculated were 7.85x10-2 mg L-1 and 0.238 mg L-1, respectively.

This will allow the determination and comparison of PEX collector kinetics and adsorption isotherm on pyrite mineral in MiliQ, drinking and sea water at pH 7.0, 7.5, 8.0, 9.0 and 10 using calcium oxide (CaO), sodium hydroxide (NaOH), ammonia buffer (0.1 mol L-1) and phosphate buffer (0.1 mol L-1) in order to evaluate and compare the use of water in the flotation process in the mining industry.

Acknowledgment: Project FONDECYT 1140206 and University of Santiago of Chile (USACH)

Keywords: Adsorption, Data Mining, UV-VIS Absorbance/Luminescence, Water

Application Code: Process Analytical Chemistry

Methodology Code: UV/VIS
Microalgae are a potential source of various valuable products and co-products for commercial applications. Cell disruption to liberate the compounds of interest is among the most critical and expensive down-stream process. Pulsed electric field (PEF) treatment is one of the most promising technologies for lipid extraction from microalgae, because of the reduction or elimination of chemical usage. As PEF is a non-thermal process, it reduces the possible damage of lipids and other substances, which potentially could be extracted from the same biomass. In addition, energy cost is much lower than other conventional lipid extraction processes. Our research focused on PEF extraction of neutral lipids from 10 strains of microalgae of economic significance. Since fatty acid methyl esters (FAMES) are considered a direct precursor to drop-in biofuels, we measured FAMES and FAME profiles in pre- and post PEF processed microalgae and the treated supernatant to determine recovery of the extracted FAMES. Our research required method development for FAME recovery from aqueous supernatant after PEF treatment. Each strain was tested under the following PEF intensities using 20 µS pulses: 0, 1, 3, 5, 10, 15, 20, 25, 30, 35 and 39 kV/cm. All strains were tested during logarithmic growth and oleaginous strains were also tested after nutrient-deprivation derived stress. The efficiency of electroporation was determined qualitatively by using Nile Red stain and fluorescent microscopy to view the extent of neutral lipid liberation between the untreated control and treated samples for all strains. Spectrophotometric scans (300 – 800 nm) were also conducted on pre- and post-treated supernatant to quantify the amount of pigments released from the microalgae cells. Finally, GC/MS with FID was used to profile and quantify the FAMES in and post-treated samples and the corresponding supernatant.

Keywords: Environmental/Biological Samples, Extraction, Fluorescence, Gas Chromatography/Mass Spectrometry
Application Code: Process Analytical Chemistry
Methodology Code: Gas Chromatography/Mass Spectrometry
Influence of Particle Size on Near-infrared Quantitative Analysis

To investigate the variation of near-infrared (NIR) spectral feature depending on particle size and its influence on accuracy of quantitative analysis, pure lactose powder/pellet and mixture powder/pellets composed of lactose/ambroxol (9-16 wt%) and lactose (5-15 wt%)/polyethylene (PE) were prepared. For the preparation of samples, the sizes of lactose were varied, while the size of PE and ambroxol was unchanged. Diffuse-reflectance NIR spectra of the samples were collected for the evaluation. As the particle size of lactose in the pellet became smaller, the peak intensity of lactose increased and an error of quantitative analysis improved. In contrast, in the case of powder samples, the intensity decreased with the decrease of particle size. To examine the observed trend of particle-size dependent spectral variations in both cases, Monte-Carlo simulation was employed to visualize the photon migration in the samples. In the simulation, porosity of sample was also considered. The reproducibility of collected spectra clearly varied with change of particle size in the samples and directly influenced on accuracy of quantitative analysis.

Keywords: Near Infrared, Process Analytical Chemistry
Application Code: General Interest
Methodology Code: Near Infrared
Data processing and modeling are key to making near infrared (NIR) technology work. NIR technology is rapidly being miniaturized and commoditized. This trend will see an explosion of applications and hence there is an urgent need to make data processing and model-building efficient.

Our research objectives are 1) to develop accurate material property prediction models using NIR spectra from portable sensors using limited data; 2) to develop robust models based on ML (machine learning) principles; 3) to accomplish training of the ML models in near real-time so as to support use in industrial production.

The materials to be characterized were obtained from 1) a pharmaceutical plant where mixtures of controlled compositions were made and 2) actual industrial process provided by our industrial partners (from food & beverage and pharmaceutical sectors). Several established portable NIR sensors were used. The true compositions were provided by using laboratory methods (like GC, HPLC, and MS). We used ML packages from WEKA & R and modified them to suit our needs.

Our breakthrough results are: 1) proved using several portable NIR sensors that they are capable of supporting the stringent needs of online analysis, 2) significantly better results in prediction accuracy by using ML when compared with traditional PLS/Linear models, 3) rapid generation of ML models by parallelizing their training on HPC infrastructure, and 4) an analysis latency of less than 1 second. In our challenging applications, we have obtained an order of magnitude improvement in accuracy and two order of magnitude increase in training the ML models.

Our results will enable the use of commodity NIR sensors (in the $100 - $20,000 range) to be applied in many industrial applications thus lowering the barrier for using NIR technology significantly.

This work is funded by the US Department of Energy.

Keywords: Data Analysis, Near Infrared, Portable Instruments, Software
Application Code: Pharmaceutical
Methodology Code: Near Infrared
It is well known that dopamine and serotonin play a very important role in biological systems and have a direct relationship with feelings and mental state, as well as physiological functions. Measurement of these molecules is of interest as an early diagnostic tool and follow-up for severe disorders such as Parkinson and Alzheimer diseases. Molecularly imprinted polymers (MIP) have been used in an increasing number of applications to selective molecular recognition. With this technique is possible to create template-shaped cavities in polymer films that are complementary in shape, size and chemical functionality to the analyte molecule used as template during the synthesis of polymers. In this work the MIP concept was used to develop selective electrochemical sensors for rapid, sequential and quantitative determination of dopamine and serotonin. The sensor consists of a gold microelectrode modified electrochemically with a thin film of polypyrrole in the presence of the neurotransmitters and subsequent over-oxidation of the film. The result is the generation of an overoxidized polypyrrole film with molecular cavities for the selective detection of the analytes. The results showed that the sensors have a lineal response (R=0.99) between 1-300 M, with a limit of detection of 1.31 and 3.83 M for dopamine and serotonin respectively. The amperometric response of the sensors was reproducible up to 3 days. A sensor for the simultaneous detection of the two molecules was also studied, the results showed a higher response for serotonin and with a sensitivity of ca. 70% of the individual sensors.
We have built the second-generation Crystal Imaging Robot using Arduino micro controller that is suitable for capturing images of multi-well plates (96-, 192-, and 288- wells) used for crystallization screening and cell culture. The imager is assembled from commercially available variable zoom USB microscope and a modular 2-dimensional stage. The software that controls the movement and captures the images is a modified open source version and the microprocessor control is built on Arduino platform. Stage movement is driven by small stepper motors and rubber belts and controlled by an Arduino microprocessor running the GRBL firmware commonly found in 3D printers. Instructions are sent through the USB serial connection to the Arduino by a python program developed in-house. The same program utilizes the python bindings for the OpenCV to perform the image acquisition. The program was developed under both Mac OS X and Linux. The imager reduces the time and fatigue involved in manual inspection while providing image history of the multi-well plates during the course of an experiment. Since the design is flexible further modifications to suit other multi-well plates and experiments are possible.

Keywords: Biological Samples, Imaging, Robotics, X-ray Diffraction
Application Code: High-Throughput Chemical Analysis
Methodology Code: Sampling and Sample Preparation
Titration is considered a fast and economical technique to evaluate a broad scope of analytes. However, getting good results can be challenging when samples are oily, difficult to dissolve, or require indirect measurement. Thermometric titration improves upon this technique by using a sensor that detects small enthalpy change – a universal property of chemical reactions. Improved Acid Number (AN) methods have already been developed and adopted by ASTM. In this talk, learn how thermometric titration works and how it will be the future for improving other difficult titration methods.

Keywords: Sample Handling/Automation, Sampling, Thermal Analysis, Titration

Application Code: General Interest

Methodology Code: Thermal Analysis
Throughout the 20th century it was widely accepted that a light microscope relying on conventional optical lenses cannot discern details that are much finer than about half the wavelength of light (200-400 nm), due to diffraction. However, in the 1990s, the viability to overcome the diffraction barrier was realized and microscopy concepts defined, that can resolve fluorescent features down to molecular dimensions. In this lecture, I will discuss the simple yet powerful principles that allow neutralizing the limiting role of diffraction1,2. In a nutshell, feature molecules residing closer than the diffraction barrier are transferred to different (quantum) states, usually a bright fluorescent state and a dark state, so that they become discernible for a brief period of detection. Thus, the resolution-limiting role of diffraction is overcome, and the interior of transparent samples, such as living cells and tissues, can be imaged at the nanoscale.


Keywords: Microscopy, Nanotechnology
Application Code: Nanotechnology
Methodology Code: Microscopy
Microelectrodes are micro-scale needle-type electrochemical sensors that can be used to measure free ion activities in soft materials of interest (e.g. biofilm). The goal of the study was to develop, characterize, and apply ion-selective microelectrodes to the vascular bundle of plants to investigate the transport of bactericide (i.e. Zinkicide) in the phloem and xylem in response to external dose changes. The ion-selective microelectrodes were fabricated by filling a glass micropipette (15 µm tip dia.) with a newly prepared ion-exchange membrane (Zn ionophore I for Zn$^{2+}$ and Na ionophore X for Na$^{+}$). The sodium microelectrodes were constructed as liquid contact ion-selective electrodes and used a 10$^{-3}$ M NaCl backfilling solution connected with an internal Ag/AgCl wire. The Zn$^{2+}$ microelectrodes were prepared in a solid contact configuration using a poly(3-octylthiophene) (POT) coated gold wire as the transduction medium and a Ag/AgCl external reference electrode. The zinc microelectrode displayed a slope of 26.7 mV/decade and a limit of detection (LOD) of 10$^{-6.8}$ M Zn$^{2+}$, while the sodium microelectrode displayed a slope of 53.3 mV/decade and a LOD of 10$^{-6.2}$ M Na$^{+}$. The zinc microelectrode sensors were tested in citrus plant extract to evaluate any potential interferences and compared to result from atomic adsorption spectroscopy (AAS). The microsensors were successfully applied to citrus seedlings to detect changes in Zn$^{2+}$ (17 mV response to a 0.001M external dose) and Na$^{+}$ ion concentrations (30 mV response to 0.1M external dose) over time. It was found that both Zn$^{2+}$ and Na$^{+}$ ions move through the xylem at 0.1 cm/day. Overall, we successfully developed Zn$^{2+}$ and Na$^{+}$ ion-selective microsensors with good stability and durability to measure ion concentrations in the vascular bundle of citrus leaves.

Keywords: Agricultural, Sensors
Application Code: Agriculture
Methodology Code: Sensors
**Abstract Title:** Quantitative Determination of Terpenes Using Solid Phase Micro Extraction

**Abstract Text:**
Terpenes can be found in a variety of plants and are responsible for their unique aromas. Due to the increase in legalization of medical marijuana there has been enhanced interest in terpenes and their therapeutic properties. Since terpenes are both volatile and semi-volatile, it can be difficult to quantify the amount of terpene in the plant. Using Solid Phase Micro Extraction (SPME) a quantitative determination of terpenes will be established for a better terpene profile.

**Keywords:** Agricultural, Quantitative, SPME

**Application Code:** Agriculture

**Methodology Code:** Gas Chromatography/Mass Spectrometry
Over half of the world's population is deficient in one or more mineral element: calcium (Ca), iron (Fe), magnesium (Mg) or zinc (Zn). Blueberry fruit is a good source of Ca, Fe, Mg, phosphorus (P), potassium (K), sodium (Na), Zn, copper (Cu), manganese (Mn) and selenium (Se). In addition to iodine (I), Na, and Se, all macro– and micronutrient elements essential for plant growth (N, P, K, Ca, Mg, S, Fe, B, Mn, Cu, Zn, Mo, and Cl), are required in the human diet. The objective of this study is to determine the level of fortification of blueberry fruits with plant essential elements. Fruit samples were taken from plant stands of the 7th year harvest of a field plot in Central Missouri on 12 June 2017. Fruit content of both essential and beneficial elements (Na, Se, and Si), and potentially toxic elements (As, Cd, Pb, and Hg) of four blueberry varieties (Bluecrop, Duke, Legacy, and Reka) were determined by inductively coupled plasma optical emission spectrometry or inductively coupled plasma mass spectrometry. The accuracy of the methods was ascertained through the analysis of certified reference materials (SRM 3287: Blueberry–Fruit and SRM 1547: Peach Leaves). The concentrations of mineral elements in fruits of the four blueberry varieties relative to daily dietary intake requirement and information on the food safety risk to humans from any of the potentially toxic elements present at concentrations above the Recommended Dietary Allowance (RDA) will be presented.
The ability to profile volatile and semi-volatile components present in Cannabis and Cannabis related products is critical as more states are approving cannabis for both medical and recreational use. The focus of this study was to develop a method to analyze the cannabidiols (CBD’s) in cannabis. CBD’s have found promising use in a variety of applications including chronic pain relief, epilepsy control, Parkinson’s, Alzheimer’s, and anti-cancer properties. 1,2

The use of direct extraction via thermal desorption has several advantages over classical extractions including increasing sample throughput, reducing the chances of volatile components being “lost” in the extraction process and eliminating the need for solvents. This study utilizes the advantages of Thermal Desorption GC/MS to identify CBD’s and other components found in Cannabis products.

Abstract Title
Analysis of Cannabidiols found in Cannabis by Thermal Desorption GC/MS

Primary Author
Ronald E. Shomo
Scientific Instrument Services

Co-Authors
Christopher Baker, John J. Manura
Computer design software and 3D print technology allow us to improve the management of agricultural pests, specifically in the capture and analysis of Asian citrus psyllid (ACP), the vector of huanglongbing (HLB), citrus greening disease. Printed traps can easily incorporate light, sound, and odor attractants to overcome potential field positioning effects and, unlike traditional sticky cards, also preserve testable specimens for rapid confirmation of the presence and spread of the HLB pathogen, [i]Candidatus liberibacter[/i] spp. The rapid prototyping available through the use of 3D print technology allows us to reduce both the time and cost required to create and assemble functional traps, within hours of gaining new information about the target pest’s behavior and for generally less than $10 per trap. Trapping of testable ACP facilitates rapid site management response to enable delimitation and potential containment of citrus greening in regions where the disease is not widespread. This presentation will share ideas and efforts regarding the benefits of integrating 3D design and printing technologies into programs for methods development and pest management research.

This work is supported by funding from Florida Department of Agriculture and Consumer Services, California Department of Food and Agriculture, and National Institute of Food and Agriculture, USDA.

Keywords: Bioanalytical, Biological Samples, Method Development, Portable Instruments
Application Code: Agriculture
Methodology Code: New Method
Raman microscopy is a nondestructive analysis technique that allows for the characterization of components of formulations with regard to chemistry, isomer and polymorphic form, particle size and distribution. A review of Raman theory, instrumentation and analysis considerations will be followed by application examples using Raman microscopy. Raman can be used for the in situ characterization of active ingredients to determine their stereochemistry, such as whether an active is in the cis or trans form of a molecule; a determination not possible with techniques such as mass spectroscopy or liquid chromatography. And Raman can also be used to identify the polymorphic form (hydrate/anhydrous, crystalline form) of a compound which will have an effect on how the active will perform when formulated with carriers and when applied in the field. Raman is a very flexible technique and can be used to analyze active and excipient materials in different forms including powder formulations, emulsions, or as neat samples. The amount of material needed for a single analysis is less than 1 nanogram making it possible to use Raman in product development to verify molecular information of a compound. With the sensitivity of Raman microscopy and utilizing automated sample stages, spatially resolved Raman data can be collected of a formulated sample producing a map of the components in a formulation. The Raman map can be evaluated for the distribution and uniformity of a product with high spatial resolution. And with that distribution, the particle size of the active material in a formulation can be determined to the micron particle size level.

Keywords: Agricultural, Analysis, Pesticides, Raman Spectroscopy
Application Code: Agriculture
Methodology Code: Molecular Spectroscopy
In recent years, increased awareness of consumer and food safety has led to a greater demand for screening of various food stuffs. This includes screening for multi residue pesticides. Like most agricultural products, pesticides are widely used for crop management and can find their way into consumer goods. It is therefore important that reliable, rapid and cost effective procedures be in place for the screening of products destined for a consumer market.

Pesticide extractions and analyses have long been in place for the food and environmental industries. Tapping into these methodologies, the usage of Pressurized Liquid Extraction (PLE) can be fitted to deliver a one step extraction and extract clean-up process for rapid GC/MS analysis of a wide array of pesticides in foods.

Extraction cells were filled with Ottawa Sand, sample aliquots, clean-up sorbents and spiked with relevant standards. Cells were sealed and loaded onto the PLE extraction system. Cells were filled sequentially with acetonitrile, pressurized and held at ambient temperature for 5 minutes. The cells were then depressurized and flushed with nitrogen to purge remaining solvent into the collection tubes. From the final extract an aliquot was transferred to a vial for LC/MS analysis.

Analysis of black and green tea, green coffee, fennel seed, astragalus root, hawthorn, and gotu kola gave excellent recoveries for approximately 150 pesticides. This method for high throughput pesticide analysis takes only 20 min which can result in processing 24 samples per hour on an 8-channel (-position) PLE system. It eliminates the need for manual extraction and potential human error involved. The automated extraction and in-cell clean up delivers fast, consistent and reproducible results.

Keywords: Food Contaminants, Gas Chromatography/Mass Spectrometry, Pesticides
Application Code: Agriculture
Methodology Code: New Method
Environmental Quantification of Polychlorinated Biphenyls, Polychlorinated Dibenzo-p-dioxins and Furans During Informal Electronic Waste Recycling in Indian Cities: Atmospheric Transport and Human Health Risk Assessment

Recent studies have shown the shift in global sources and source regions of certain hazardous organic compounds like polychlorinated biphenyls (PCBs), polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) due to a final transition in the life-cycle towards the re-cycling and disposal stages paralleled by significant reductions in atmospheric burdens within the regions (i.e., the developed nations) where they have been produced and extensively used. Increasing demand for electronic equipment in domestic India following rapid economic developments, inevitably lead to large amounts of domestic electronic waste (e-waste) when these items are discarded. The major objectives of this study were to elucidate the emission and atmospheric transport of PCBs, PCDDs and PCDFs assess the potential risk posed by these hazardous contaminants to the human health. HRGC-HRMS results from the core crude e-waste recycling sites in India showed that the highest level of furans was from an acid leaching site in New Delhi (5100 pg/g) where recovery of precious metals from the crude e-waste takes place. Interestingly, dioxin like PCBs were extremely high for Chennai and Mumbai ranging between 1100-436865 pg/g, with the maximum level at a typical metal recovery site in Chennai. HYSPLIT and FLEXPART trajectory models showed that the elevated temperatures encountered in tropical climate have profound implications for the atmospheric transport of these semi-volatile organic contaminants away from source regions. Significant correlation exist in Indian cities (R² = 0.64; p< 0.01) between TEQ and 33PCB concentrations in soil and atmosphere. In addition NEQ values were found to be strongly significant with 33PCBs (R²=0.99; p<0.01). Crude e-waste recycling may pose serious impact on human health.

Keywords: Environmental Analysis, Environmental/Air, Environmental/Biological Samples, Environmental/Soils

Application Code: Environmental

Methodology Code: Gas Chromatography/Mass Spectrometry
Environmental Comparative Studies on Intraparticle Diffusivity of Some Selected Divalent Metal Ions Removal from Polluted Water Samples Using Different Waste Biomass

Waste management is one of the means of controlling environmental pollution. This work emphasizes on the comparative studies of intraparticle diffusivities of Cd²⁺, Ni²⁺ and Pb²⁺ onto unmodified and mercaptoacetic acid modified fluted pumpkin seed coat, oil bean seed shell and kolanut pod husk using contact time as an important experimental parameter. Adsorption was carried out in a batch process at various contact time with initial metal ions concentration of 100 mg/l using 1 g of 250 µm size of the wastes biomass at a temperature of 25 ⁰C and pH of 7.5. Generally, the extent of adsorption increases with decrease in time for the unmodified wastes biomass and increase in time for the modified wastes biomass, maximum value given as 99.99 mg/l. Both Mckay & Poots and Weber & Morris intraparticle diffusion models fitted the experimental data and confirmed the process to be particle diffusion controlled. R² values, indicated good correlation of some of these metals with both unmodified and modified wastes biomass.

Keywords: Adsorption
Application Code: Environmental
Methodology Code: Chemical Methods
Persistent organic pollutants (POPs), in particular organochlorine insecticides, have been and are still used in Nigeria on a widespread scale for over five decades. Since streams, rivers and other surface water bodies receive runoffs from land, residues of various chemicals used on land end up impacting on their quality as contaminants. The Bight of Benin in the Lagos area (3o, 00’ - 4o, 30’E and 5o, 30’ – 6o, 30’N) in South Western Nigeria receives inputs from upland streams and rivers through the Lagos and Lekki lagoons. There is also a lot of fishing and shipping activity in the area which extends into the Atlantic Ocean. Twelve species of marine fish and one of sea snails caught from the area between 1986 and 1987 were analyzed for their content of POPs (specifically, organochlorine compounds- OCs) as a means of estimating the area’s contamination status with these compounds. The POPs detected in these fish samples and their concentrations in ng/g include: aldrin (0.45-7.78; mean 2.31); DDT and metabolites (0.56-32.7, mean 7.12); \( [\alpha] \)-HCH (ND – 0.17, mean 0.12); \( [\delta] \)-HCH (ND – 0.73, mean 0.73); Heptachlor (ND – 0.54, mean 0.16); Heptachlor epoxide (ND – 1.76, mean 0.68); Endosulphan (ND – 1.65, mean 1.18); Lindane (0.05 – 0.55, mean 0.27) and PCBs (0.05 – 0.35, mean 0.18). Of these aldrin, lindane, \( p,p' \)-DDE and PCBs gave 100% detection response in all the samples. The DDT/PCB ratio suggests that the contamination of the biota samples by the presence of the residues found is largely of agricultural origin.

Keywords: Environmental/Water, Monitoring, PCB’s, Pesticides
Application Code: Environmental
Methodology Code: Gas Chromatography
Perchlorate has been used as an oxidizer in rockets, munitions, and fireworks since the 1950s. It has been found to cause thyroid dysfunction, and has been linked to tumors in humans. Perchlorate is regulated under the Safe Drinking Water Act (2011). Massachusetts and California have established standards for drinking water of 2 μg/L and 6 μg/L respectively.

U.S. EPA Method 332.0 — Ion Chromatography with Suppressed Conductivity and Electrospray Ionization/Mass Spectrometry is one of the most sensitive and robust characterization methods available for perchlorate determinations. Mass spectrometry (MS) provides lower detection limits in high-ionic-strength matrices than conductivity detection alone. These low detection limits are achieved without sample preparation.

Our study updates the IC-MS method published in U.S. EPA Method 332.0 for determination of perchlorate in environmental waters. The method uses a Thermo Scientific™ Dionex™ IonPac™ AS20 column set, on a recently introduced compact IC system coupled with a recently introduced single quadrupole mass spectrometer. The selectivity of the mass spectrometer allows the quantification of perchlorate in high-ionic-strength samples at well below currently enforced action levels. Ionization improvements to the electrospray source eliminate the need to add organic solvent to enhance detection. Method detection limit (MDL) values in deionized water are 20-60 ng/L, and MDLs in high-ionic-strength matrix are 30-60 ng/L. The calibration curves for perchlorate in high-ionic-strength matrix at 101 m/z over the range of 125-5000 ng/L using the internal standard and external methods showed good linearity with the coefficient of determination being 0.9993, and 0.9998 respectively.
Environmental Determination of Inorganic Anions in Wastewater Using Capillary Ion Chromatography

Ion chromatography (IC) is a well-accepted technique for the monitoring inorganic anions in waters, including surface, ground, drinking, and wastewaters and IC methods are specified by regulatory agencies for environmental water analysis. Typically these methods use standard or microbore columns. Scaling down from standard bore (4 mm i.d.) to capillary format (0.4 mm i.d.) brings many benefits to the analyst, including low eluent consumption and low waste generation. With such low mobile phase consumption, the system can remain running and be ready for analysis. Unfortunately the smallest available injection valve internal injection loop (400 nL) required that many water samples, including wastewater, be diluted as to not overload the IC column. This dilution is not needed for standard bore IC. This presentation shows how a new injection valve with a 100 nL internal injection loop can be used for wastewater and municipal drinking water and anion analyses without sample dilution. We show the method calibration, determine the detection limits, and measured the method’s precision and accuracy. The calibration was linear over a wide range for the standard anions (e.g. 0.2 to 300 mg/L for chloride) and we found that the method was both accurate (86–106%) and precise (RSD < 3.2%) for common anions such as fluoride, chloride, nitrite, sulfate, nitrate, and phosphate with this low injection volume.

Keywords: Capillary Ion Analysis, Chromatography, Environmental Analysis
Application Code: Environmental
Methodology Code: Liquid Chromatography
The Characterization and Identification of Benzotriazole Derivatives in Wastewater by GCxGC-TOFMS

One of the main sources for contaminants of emerging concern (CECs) in the environment are wastewater treatment plants (WWTPs). CECs, including pharmaceuticals, personal care products and endocrine disrupting compounds, are present in post-treatment waters due to inefficient removal at the WWTP. The Penn State WWTP utilizes tertiary treatment methods, chlorine disinfection and irrigation of crop land with effluent water, to further remove the CECs. In this study, pre-treatment influent, post-treatment effluent, spray field irrigation effluent and groundwater samples were analyzed for CECs using Comprehensive Two Dimensional Gas Chromatography with Time of Flight Mass Spectrometry (GCxGC-TOFMS). This method allowed for efficient separation of the complex water samples revealing a variety of CECs. By far the most prevalent were those of the benzotriazole compound class, used as corrosion inhibitors. These compounds were not removed in the WWTP and chlorinated byproducts were formed in the tertiary treatment step. This finding led to further study including synthesis and identification of the chlorinated compounds, for which commercial standards do not exist.

Keywords: Environmental/Waste/Sludge, Environmental/Water, Gas Chromatography/Mass Spectrometry, Identification
Application Code: Environmental
Methodology Code: Gas Chromatography/Mass Spectrometry
Environmental Retention Modeling and Retention Time Prediction in Comprehensive Two-Dimensional Gas Chromatography

A model for predicting retention times in comprehensive two-dimensional gas chromatography (GCxGC) is presented. Retention times are determined thermodynamically using the classic two parameter model. A correction factor is applied which significantly improves retention time predictions in the second dimension column. Separations are modeled with improved accuracy compared to more complex GCxGC retention time prediction models, while also requiring less data collection.

Keywords: Gas Chromatography, Gas Chromatography/Mass Spectrometry, GC, GC-MS
Application Code: Environmental
Methodology Code: Separation Sciences
Dirt and gravel roads tend to kick up a lot of dust, especially during the drier summer months. Typically, an oily dust suppressant is applied to roads to prevent excess dust. In Pennsylvania, oil and gas extraction has generated large amounts of oily and briny wastewaters. A legally allowed disposal option for oil and gas wastewaters is beneficial reuse as a dust suppressant on local dirt and gravel roads. We obtained samples of oil and gas wastewaters from townships in Pennsylvania that use this practice for dust suppression. As part of a larger study which analyzes the wastewaters for metal concentrations and radioactivity, we utilized comprehensive two-dimensional gas chromatography (GCxGC) coupled to time-of-flight mass spectrometry (TOFMS) to (1) characterize the oil and gas wastewaters for organic content, (2) perform volatilization experiments to simulate contaminant release to air after wastewater application to roads, and (3) assess risk of surface water contamination though rain water leach experiments. Overall, this study was aimed to understand the environmental impact of using oil and gas wastewater as a road treatment.

Keywords: Environmental Analysis, Fuels\Energy\Petrochemical, Gas Chromatography/Mass Spectrometry, Tim
Application Code: Environmental
Methodology Code: Gas Chromatography/Mass Spectrometry
The potential of total excitation-emission fluorescence microscopy combined with multiway chemometric analysis was investigated for the nondestructive analysis of textile fibers. Undyed textiles such as Acrylic 864, Nylon 361 and Cotton 400 were pre-dyed with Basic Green 4 (BG4), Acid Yellow 17 (AY17) and Direct Blue 1 (DB1) dyes, respectively; and were exposed to humid (Florida) and dry (Arizona) weathering conditions for three, six, nine and twelve months. After every three-month interval, ten fibers were uniformly sampled, and excitation-emission matrices (EEMs) were obtained using fluorescence microscopy. In addition to the loss of fluorescence emission intensity over the twelve-month period, changes in fluorescence emission spectral profiles were observed by comparing 2D spectra and 3D EEMs obtained from exposed and non-exposed fibers. Most rapid degradation of textile dyes occurred under dry climate. Discriminant unfolded partial least-squares (DU-PLS) – a second-order multivariate calibration method – was used to statistically determine the weathering effects on the spectral features of the selected fibers. In all cases, this algorithm was unable to differentiate non-exposed acrylics from exposed acrylic fibers. DU-PLS was able to differentiate non-exposed cotton and nylon fibers from exposed fibers to Florida and Arizona weathering conditions. It was possible to determine the period of exposure to either Florida or Arizona conditions, and it was also possible to discriminate between fibers exposed to Florida or Arizona weathering conditions for the same period of time. These results will provide a foundation for future studies towards a non-destructive approach capable of providing information on the history of the fiber.

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Keywords: Chemometrics, Fluorescence, Microscopy, Spectroscopy
Application Code: Environmental
Methodology Code: Fluorescence/Luminescence
Due to the complexity of oil contaminated sites, the unambiguous identification and quantitation of environmental pollutants often requires the sequence of high-performance liquid chromatography (HPLC) and gas chromatography-mass spectrometry (GC-MS). A classic example is the analysis of the sixteen polycyclic aromatic hydrocarbons included in the priority pollutants list of the U.S. Environmental Protection Agency (EPA-PAHs).

This presentation focuses on the photoluminescence spectroscopy of polycyclic aromatic sulfur heterocycles (PASHs). Since focusing on EPA-PAHs alone can lead to drastic underestimations of potential toxic effects of oil spills, a strong case can be made for including hetero-aromatic compounds in risk assessments of contaminated sites. Due to the asymmetry imposed by the heteroatom, PASHs exist in an even greater variety of chemical structures than PAHs. Therefore, the number of PASHs isomers with the same molecular weight (MW) can be extremely large, which increases the difficulty of separation and identification by chromatographic approaches.

Herein, we demonstrate the capability to differentiate individual PASHs isomers of MW 234 g mol⁻¹ via vibrational spectroscopy at liquid nitrogen (77 K) and liquid helium (4.2K) temperatures. We present phosphorescence spectra with fingerprint information for specific isomer identification at the parts-per-billion (ng.mL⁻¹) concentration levels. The relatively long phosphorescence decays of PASHs facilitate the time discrimination of the strong fluorescence background often observed from environmental samples. Characteristic phosphorescence lifetimes provide and additional qualitative parameter for the unambiguous and accurate determination of MW isomers in oil spill samples.

Acknowledgement:
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Keywords: Environmental, Luminescence, Molecular Spectroscopy, Temperature
Application Code: Environmental
Methodology Code: Fluorescence/Luminescence
Environmental Extraction and Quantification of Phosphorus in Lake Sediments

Understanding the bioavailability of phosphorus (P) in freshwater lakes is important to controlling algal blooms and meeting environmental regulation requirements. The chemical speciation of P in lake sediments plays an important role in determining whether P will remain buried or diffuse back into the water column and become bioavailable for algae. A chemical sequential extraction technique for sediment P will be presented with quantification by ion chromatography (IC) and inductively coupled plasma mass spectrometry (ICP-MS). Changes in the seasonal P speciation within lake sediments were determined including organic, precipitated, and adsorbed forms of P. Lake water column depth and sediment type played an important role in P speciation and concentration. Shallow sediments contained a higher fraction of adsorbed P in sandy, silty grains compared to higher fractions of organic P in deeper, more organic-rich sediments. Additionally, reduction/oxidation reactions within lake sediments played an important role in P chemical speciation. Iron and manganese concentrations determined from porewater ICP-MS measurements demonstrated a redox active zone within the top 4 cm below the sediment-water interface. Reduction of iron (III) oxyhydroxides to iron (II) oxides released adsorbed orthophosphate from iron surfaces filling sediment pore spaces with bioavailable P, which was not observed to be released back into the water column. The occurrence of aluminum phosphate solid-phase minerals in x-ray diffraction (XRD) patterns of sediments suggest that released P from iron surfaces re-adsorbed to non-redox active aluminum mineral surfaces or precipitated as aluminum phosphate minerals.

Keywords: Environmental Analysis, Environmental/Water, Extraction, ICP-MS
Application Code: Environmental
Methodology Code: Separation Sciences
Environmental Analysis of Perfluorinated Compounds in Water Using Automated Solid Phase Extraction

Perfluoralkylated substances is a general term used to describe substances which are largely comprised of, or contain a perfluorinated or polyfluorinated carbon chain moiety of, F(CF2)n- / F(CF2)n-(C2H4)n . PFOS and other perfluorinated compounds are widely used in industrial and consumer applications including stain-resistant coatings for textiles, leather, and carpets, grease-proof coatings for paper products used in food, firefighting foams, mining and oil well surfactants, floor polishes, and insecticide formulations. In recent years, there has been increasing concern over levels of perfluorinated and polyfluorinated chemicals, such as PFOS (perfluorosulfonate) and PFOA (perfluoro-octanoicacid), in the global environment and their fate and possible adverse effects in the environment.

This study demonstrates the first automated solid phase extraction system made specifically for PFC extraction and concentration. Especially effective at reducing background contamination, extraction and concentration of aqueous samples takes less than two hours.

500 mL water samples were spiked with 25 uL of a 1 ug/mL PFC standard solution. Samples were then loaded onto the PFC SPE system and passed across an HLB Plus 225 mg cartridge under -12 psi vacuum. After loading, bottles were rinsed with 25 mL of water and loaded onto the cartridge under negative pressure. The cartridges were dried using nitrogen, and then subsequently eluted with methanol. The extracts were concentrated to 500 uL, after which internal standard was added. The samples were diluted to a final volume of 1 mL for LC/MS analysis.

Recoveries of spiked standards varied between 75-125% for a total of fourteen different PFCs. Background concentrations varied from 0.01 ng/L for PFPeA and PFDoA to 0.2 ng/L for PFOA. The SPE system for PFCs produced reliable, reproducible results for PFCs in water. The system has very low background PFC allowing for analysis of samples without significant interference.

Keywords: Automation, Liquid Chromatography/Mass Spectroscopy, Solid Phase Extraction
Application Code: Environmental
Methodology Code: Liquid Chromatography/Mass Spectrometry
Tap water samples were collected from all 76 schools in the Winston-Salem/Forsyth County Schools (WSFCS) district. First draw samples were taken from cafeteria sinks and water fountains. ICP-MS/MS was used to determine the concentrations of eight toxic elements: As, Cd, Cr, Cu, Pb, Sb, Se and Tl. The results were compared with maximum contaminant levels (MCL) set by the EPA. The method’s accuracy was checked by analyzing a certified reference material from NIST, with recoveries ranging between 87 and 98%. Limits of detection (LOD) for all eight elements were below the MCLs (LOD between 4 - 300 ng/L). All samples from currently active schools tested below the MCL value set for each element (e.g. Pb values were all <5.01 µg/L). One school, vacant for approximately two years, showed a spike in Pb, with a value of 44.11 µg/L. A flushing procedure was implemented to reduce the Pb concentration at that tap. After 5 min of flushing, Pb was reduced to below 1 µg/L. A study was performed to evaluate the effect of water stagnation during the summer break on Pb concentrations in the school’s drinking water supply. Four schools, selected according to the age of the building, Pb concentration during the school year and activity level over the summer break, were tested after 53 days of drinking water stagnation. The same taps tested during the school year were re-tested after the summer break at five points throughout a 2 h flush period. A school with a previous Pb concentration of 2.33 µg/L during the school year, for example, reached a value of 4.24 µg/L after the 53-day stagnation period. After 5 min of flushing, the concentration at that tap reduced to below the LOD for Pb. Water stagnation and the age of the building do increase the chance of higher elemental concentrations, especially Pb. Based on the results of this study, a simple 5 - 25 min flushing procedure is recommended prior to drinking water use of unoccupied buildings.

Keywords: Elemental Mass Spec, Environmental/Water, Tandem Mass Spec, Trace Analysis
Application Code: Environmental
Methodology Code: Atomic Spectroscopy/Elemental Analysis
Environmental

Determination of Iron and Manganese in Drinking Water Samples by TXRF Spectrometry

Quantitative measurements of some ions, including iron and manganese in drinking water is a routine. Besides higher concentrations of these ions does not show toxic properties, they can generate color, a secondary aesthetic parameter, affecting the appearance and palatability of the water. Brazilian legislation establishes the limit of 0.3mg L-1 for Fe and 0.1mg L-1 for Mn.1 Besides Atomic Absorption Spectrometry (AAS) and Inductively Coupled Plasma Optical Emission Spectrometry (ICP OES) have been routinely applied on this kind of analysis, Total Reflection X-ray Fluorescence spectrometry (TXRF) show to be a very interesting alternative since it provides a fast and easy sample preparation, low analytical operation and low maintenance costs.2 In this way, this work deal with method development for Fe and Mn quantitative measurements in drinking water by TXRF. Measurements were performed using TXRF spectrometer S2 PICOFOX (Bruker), equipped with an air cooled low power X-ray tube (Mo target). For internal calibration, 1mL of sample was mixed with 20uL of Ga standard solution (10mg L-1). The accuracy was checked by adding 0.3mg L-1 of Fe (102815, Absolute Standard, Inc.) and 0.1mg L-1 Mn (P01081, Ultra Scientific) onto drinking water samples. Recovery about 99.7% (0.299+0.041 mg L-1) for Fe and 103% (0.103+0.008 mg L-1) were obtained indicating good accuracy of proposed method. The limits of detection (LOD) were 0.04 and 0.02mg L-1 for Fe and Mn respectively, showing adequate values for both elements. Considering all this, we can conclude that TXRF spectrometry showed to be a very interesting alternative to be used in Fe and Mn measurement on drinking water samples analysis.

References:
1 BRASIL. MINISTÉRIO DA SAÚDE. Portaria nº 2914 de 12 de dezembro de 2011.

Keywords: Metals, Water, X-ray Fluorescence
Application Code: Quality/QA/QC
Methodology Code: X-ray Techniques
**Session Title**: Environmental Analysis, Environmental/Water, GC-MS, Sample Preparation  
**Methodology Code**: Environmental  
**Application Code**: Sampling and Sample Preparation  

**Abstract Text**  
USEPA method 8270 is a workhorse method for a large group of semivolatiles in groundwater, leachates and other water samples that may be required to monitor waste disposal. As such, a comprehensive multi sample extraction procedure that results in less extraction solvent while covering a full range of compounds is desired. This paper will discuss method 3535 Solid-Phase Extraction (SPE) utilizing the one-pass method. This will be demonstrated by passing an acidified sample through a SPE disk and carbon cartridge with a variety of sample matrices. Since the water is not subsequently basified and extracted again, the possibility of forming emulsions is virtually eliminated. The QC requirements of the analysis method will be demonstrated and the ability of disk SPE to handle samples with particulates will be explored.
As exploratory analytical scientists we attempt to further the investigation of unique and otherwise unfamiliar environments. Time and again approaching these unexplored regions requires the development of new sampling technologies to best address site specific challenges. Particularly much deep sea sampling is still performed by bulk collection of large volumes of by means of an ROV submarine. These bulk samples require much space on the ROV and may not preserve otherwise unstable compounds.

 Appropriately, the presented work outlines the design, validation, and deployment of SPME based samplers for the untargeted characterisation of underwater environments. These samplers employ hydrophilic lipophilic balanced (HLB) particles which have been coated onto recessed stainless steel bolts by use of polyacrylonitrile (PAN) glue. 6 coated bolts are then inserted into a self-sealing Teflon bodied sampler designed to preserve extracted compounds for extended periods. To verify this stability, 3 samplers were deployed on-site at a waste-water treatment facility outflow pipe via kayak. Post-sampling, the samplers were stored using 3 storage conditions including: A: immediate desorption, B: 3 days at 23 °C, C: 14 days at 23 °C and D: 14 days in a -80 °C freezer. All screws tested were statistically indistinguishable when analyzed using principle component analysis (PCA). Finally in a cutting edge application these samplers were tailored for use on a ROV submersible and employed for the on-site sampling of hydrothermal vents at 3 locations along the Pacific Rim with, 3 corresponding control extractions also performed from ambient waters away from these vents as to differentiate features. Separation and analysis of all samples were performed using a HPLC equipped orbit-trap mass spectrometer and 100’s of statistically unique features could be determined from the vents by use of PCA techniques.

**Keywords:** Environmental/Water, Robotics, Sampling, SPME

**Application Code:** Environmental

**Methodology Code:** Sampling and Sample Preparation
Carbon Nanotubes have attracted interest for over the last decade due to good electrode properties and petite size. Due to these positive features carbon nanotubes (CNT) modified electrodes have expanded into uses as biosensors to detect medicinal and environmental compounds of interest with electrochemistry. CNTs have opened a new era in material science and these materials are used to enhance conductivity while using selective polymers as electrochemical sensors to detect common neurotransmitters (1,2-dihydroxybenzenes) to heavy metals such as lead and cadmium. These CNT modified electrodes: CNT-polyvinyl alcohol (PVA) electrode to detect 1,2-dihydroxybenzenes to phenol in the presence of ascorbic acid, poly-3-hexythiophene (P3HT) modified CNT electrode to detect heavy metal lead and polystyrene sulfonate (PSS) modified CNT electrode to detect lead and cadmium simultaneously with prior separation will be shown. Our understanding of CNT dispersed with certain polymers to selectively detect the chemical of interest and provided efficient hole or electron transport to successfully detect the compounds of interest. These inquiry-based educational research experiences have been beneficial in allowing expansion of content knowledge in the applications of CNTs while utilizing electrochemistry techniques such as cyclic voltammetry, differential pulse voltammetry and square wave anodic stripping voltammetry. This presentation will further illustrate some novel aspects of integrating CNT -PSS with nanoparticle incorporation to possibly further enhance the electrocatalytic activity of the electrode surface for heavy metal detection. In addition, our educational perspective from participating students content knowledge gains related to the CNT modified electrode labs from pre- and post-test assessments will be shared to show our overall findings with these various lab modules.
Degradation kinetics of amines is important for the design of absorber and stripper for post-combustion CO2 capture. Current investigation evaluates the degradation kinetics of oxidation of PZ and DEA blend under absorber conditions. Oxidation experiments of 3.2 m DEA/0.8 m PZ were conducted using a low-pressure stirred tank semi-batch glass reactor. Two gas compositions (%), 50/10/40 and 90/10/0 (O2/CO¬2/N2) of gas at constant flow rate of 100 ml/min were used at temperatures 40-60 °C. Partially degraded samples were analyzed using GC-MS and GC-FID for identification of degradation compounds and quantification of parent amine loss. Experimental data is used to model the rate of degradation of PZ and DEA. Experimental results suggest that loss of PZ is very high in presence of DEA and it increases as O2 partial pressure and temperature are on rise. Degradation rate of DEA is decent and similarly its degradation rate also increases on the increase of temperature and O2 partial pressure. Degradation rate models are found to fit experimental data appropriately. Coefficients of the models show that loss of PZ and DEA are a function of Oxygen concentration. However, rate of degradation of PZ is highly synergized by the presence of DEA and concentration of PZ may play an opposed role in the degradation of DEA.

Keywords: Air, Gas Chromatography, GC-MS, Separation Sciences
Application Code: Environmental
Methodology Code: Gas Chromatography/Mass Spectrometry
Environmental Agrochemical Metabolite Identification Using Advanced UPLC and Metabolite Generation Techniques

The identification of metabolites in complex environmental and biological samples presents great challenges, due to the low levels and the complexity of the matrices. To address these challenges within our field of crop protection regulatory sciences, a semi-automated ultra-performance liquid chromatography (UPLC) method development strategy was integrated within the metabolite isolation and identification process, which significantly improved the efficiency and sample quality for successful metabolite identifications in complex samples. The versatile method scouting approach coupled the Agilent 1290 Infinity Series to UPLC fraction collector for rapid column screening, and mobile phase/gradient optimization to achieve the best separation that will resolve co-eluting components and interfering matrices. This presentation will also highlight the metabolite generation techniques such as utilization of microbial and microsomal systems to facilitate the metabolite identification and gain early understanding of the metabolite profile. Several case studies will be presented to show the challenges encountered and the solutions utilizing the UPLC method scouting – fractionation strategy in conjunction with the advanced high resolution Mass Spectrometry and NMR techniques.

Keywords: Environmental/Biological Samples, Identification, Method Development, Separation Sciences
Application Code: Environmental
Methodology Code: Sampling and Sample Preparation
Citizen science is defined as scientific project conducted wholly, or in part, by amateur or nonprofessional individuals. This talk discusses the development of tools and methods to evaluate the effectiveness of citizen scientist participation in monitoring contaminants of emerging concern (CECs) such as neonicotinoid pesticides, pharmaceuticals, personal care products, food additives etc. in drinking water. We are using solid phase extraction (SPE) and thin layer chromatography (TLC) and combining them with a smartphone based app (named ChemHunter) and advanced web based data informatics and analysis tools to seamlessly identify and quantify CECs. Its packaged as a user-friendly kit so that analysis can be performed in the field itself, at a citizen scientist’s home water faucet, or at a nearby river, lake or a well. These types of field SPE-TLC kits are already available commercially to the law enforcement community for detecting illegal narcotics drugs but haven’t been packaged and made accessible to a citizen scientist or a volunteer based water monitoring groups till now and we believe that it has the potential to change the way large scale CECs monitoring is conducted.

**Keywords:** Environmental/Water, Pesticides, Solid Phase Extraction, Thin Layer Chromatography

**Application Code:** Environmental

**Methodology Code:** Portable Instruments
Environmental Microalgae as Embedded Environmental Monitors

In marine ecosystems, microalgae are important components as they transform large quantities of inorganic compounds into biomass and thereby drive changes in environmental chemistry. Of particular relevance is phytoplankton’s sequestration of atmospheric carbon dioxide, a greenhouse gas, and nitrate, one cause of harmful algal blooms. On the other hand, microalgae sensitively respond to changes in their chemical environment, which initiates adaptations of their chemical composition and physical parameters. Analytical methodologies were developed in this study that utilize microalgae’s adaptation as a novel approach for in-situ environmental monitoring.

To analyze the chemical composition and physical parameters of live microalgae cells, FTIR-ATR spectroscopy has been employed. By means of time series of spectra, the formation of biomass can be monitored and it has been shown that nutrient availability has a considerable impact. Since biomass formation is governed by several biological parameters, this enables studies of the chemical environment’s impact on physical parameters of the cells.

Moreover, the spectroscopic signatures of microalgae grown under different carbon dioxide and nitrate mixtures have been analyzed with a novel nonlinear modeling methodology coined ‘Predictor Surfaces’ that relates the nonlinear responses of the cells to their chemical environment. This approach was used to measure carbon dioxide concentrations in the atmosphere above the algae cultures as well as dissolved nitrate concentrations within the growth medium. The achieved precision of concentration predictions were a few percent of the measurement range. This methodology will open new approaches to study the link between concentration levels of an ecosystem and the biological impacts.

Keywords: Biological Samples, Chemometrics, Environmental, FTIR
Application Code: Environmental
Methodology Code: New Method
Chlorination of seawater is considered as one of the most effective and least expensive process to control biofilm and mold development in industrial pipelines. However, these large releases of chlorine in the environment may cause the formation of various classes of chloro-brominated by-products, when residual chlorine react with natural or anthropogenic organic matter already present in seawater. Two sea sampling campaigns -representing two hydrological conditions- carried out in an industrialized semi-enclosed bay (Gulf of Fos, Mediterranean Sea, France) have enabled to spike seawater and air samples, spread throughout this 42 sq km industrialized bay (24 stations × 2 depths× 2 campaigns). These samples were used for the analysis of total halogenated compounds (EOX), and their speciation (such as trihalomethanes (THM), haloacetonitriles (HAN), halophenols (HP) and a new class of disinfection byproducts [i] i.e [/i] halobenzoquinones (HBQ)).

Different sample preparation techniques were tested and specific analytical methods were developed for these seawater samples. THM, HAN and HP in water samples were analyzed using a gas chromatography with an electron capture detector (GC-ECD). HBQ in seawater were analyzed using ultra performance liquid chromatography-quadrupole time-of-flight mass spectrometer (UPLC/Q-ToF-MS). Air samples were collected with canisters and analyzed on-site (by use of a mobile laboratory equipped with a Proton Transfer Reaction-Time of Flight-Mass Spectrometer (PTR-TOF-MS)). This work showed that the levels of chlorination by-products, whatever in seawater or in air, varied considerably from one compound to another, and depending on the location of the samples.

Acknowledgements: This work was included in the project “Fos-Sea” funded by the French Research Agency (ANR-16-CE34-0009). Maxime Verlande thanks The Conseil regional Provence Alpes Côte d'Azur and the Institut Ecocitoyen pour la Connaissance des Pollutions for his doctoral scholarship.

Keywords: Contamination, Environmental/Air, Environmental/Water, Time of Flight MS
Application Code: Environmental
Methodology Code: Liquid Chromatography/Mass Spectrometry
In the automotive industry, regulatory requirements applying to qualification type testing procedures have been upgraded since the introduction of new gasoline mixes on the market. According to several industry codes and standards, evaporative losses from gas handling and storage system have to be tested and quantified for vehicle certification. Sealed Housing for Evaporative Determination (SHED) is used to measure evaporative emissions. Photoacoustic spectroscopy (PAS) is approved by US government as a measurement method for SHED (regulation 40 CFR Part 1065.269).

Components of interest are ethanol, methanol and refrigerant freons (R-134a and HFO-1234yf). For example, E10 gasoline (10% ethanol) is used, ethanol vapors escape from the tank and permeate through plastic materials (e.g., fuel tanks and fuel hoses). The US EPA prescribes a conservative 1.08 correction factor on conventional FID measurements to account for ethanol emissions. This is unless its vapor fraction is directly measured by advanced instruments. Ethanol is supervised by regulations due to toxicity to environment. Various methods are approved for the ethanol measurement (e.g. GC-FID or PTR-MS) but PAS is currently the most practical one. Gasera addresses the SHED monitoring need with laser-based photoacoustic detection1, 2. Low-ppb level detection limits for can be achieved for all components with a combination of widely tunable high power external cavity quantum cascade laser (EC-QCL) and patented ultra-sensitive cantilever sensor. High resolution of the laser source enables simultaneous and selective measurement of all the four components of interest. The achieved performance is demonstrated against EPA regulation.


Keywords: Biofuels, Gas, Gasoline, Photoacoustic
Application Code: Environmental
Methodology Code: Molecular Spectroscopy
Humic acids (HA) are heterogeneous macromolecular organic compounds, which ubiquitously exist in soil, river, etc., and have significant effect on the mobility of metal ions in environment due to their affinity to metal ions. However, the interaction between HA and metal ions are still obscure since there is no method to investigate what kinds of HA strongly bind with metal ions. Here, we report two dimensional polyacrylamide gel electrophoresis (PAGE) for separation of HA and detection of metal ions bound to HA, combined with UV-Vis spectrometry and excitation-emission matrix (EEM) spectrometry-parallel factor analysis (PARAFAC) method. Using this method, the relationship between HA-bound metal ions, molecular weight (MW) of HA, HA concentration, and fluorescent HA species is accessible for the first time. Metal-HA complexes were separated by first dimensional PAGE, followed by elution of HA-bound metal ions in each MW fraction. Then, the metal ions were derivatized into fluorescent probe complexes, prior to second dimensional PAGE for determination of metal ions. In addition, each fraction separated in the first PAGE was electroeluted to obtain HA solutions, followed by UV-Vis and EEM-PARAFAC. Applying this method to peat soil HA complexed with terbium and uranyl ions, high concentration of terbium was detected in the high MW region. The terbium concentration increased with increasing MW (8-55 kDa). This result implies that the aggregation of HA was mediated by multidentate terbium, which generally has 8-10 coordination sites. Comparing to the result of terbium-HA complexes, most uranyl ions which provide 4-6 planer coordination sites, were detected in low MW region (< 4 kDa). This fact suggests that different coordination modes of metal ions cause different interaction and aggregations. In addition, it was found that a protein-like fluorescent component observed by EEM-PARAFAC, which localized in the low MW region, was related to the complexation of uranyl.

Keywords: Environmental/Soils, Fluorescence, Separation Sciences

Application Code: Environmental

Methodology Code: Separation Sciences
Environmental Metal Accumulation in Crayfish from Streams and Cultured Ponds in Missouri

Freshwater crayfish are widely consumed worldwide and are popular culturally in many traditions[superscript 1]. Previous works reported the accumulation of high levels of metals in crayfish [superscript 2]. This study determined the concentrations of 13 elements (As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Sn, V, and Zn) in crayfish species from three cultured ponds (n=154; Busby Farm, Jefferson City; Crystal Lake Fisheries, Ava; and Ozark Fisheries, Stoutland) and two wild sources (n=108; Brushy Creek, Oates; and Pike Creek, Poplar Bluff) in Missouri. The analysis was performed by inductively coupled plasma – optical emission spectrometry. Tissue samples (gills, hepatopancreas, exoskeleton, and abdominal muscle) were mineralized by acid microwave digestion. Method accuracy was ascertained through analysis of certified reference materials (DORM-2: Trace elements in dogfish muscle, and TORT-2: lobster hepatopancreas reference material for trace metals). The goal of this work is to evaluate and compare the qualities of crayfish from wild sources and cultured ponds. To accomplish this goal, data on the relative concentrations and distribution of metals in various tissues, bioaccumulation factors, and compliance with regulatory thresholds will be discussed.


Acknowledgements
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Keywords: Atomic Spectroscopy, Biological Samples, Elemental Analysis, Food Contaminants
Application Code: Environmental
Methodology Code: Atomic Spectroscopy/Elemental Analysis
An extensive amount of biomolecules has been studied for heavy metals removal due to the great world interest in reducing the environmental concentrations of these elements. Among the contaminants, mercury (Hg) is listed as one of the most worrisome chemical elements for public health. Bixin, a major component of the seeds from urucum (Bixa Orellana), is a hydrophobic, ionizable, reductive and non toxic apocarotenoid. This compound can be a promising alternative for the removal of organic and inorganic contaminants. In the present work bixin was incorporated on poly tetra fluoroethylene (PTFE) filters by the drop coating technique, and after then placed in contact with Hg (II) solutions. To determine the residual Hg in solutions it was used a Direct Mercury Analyzer (DMA 80, Milestone) based on the thermal decomposition of the sample and detection by atomic absorption spectrometry. The sorption capacity of Hg+2 by the bixin films was evaluated changing the parameters pH, time, temperature, concentration and nature of salts in the solution. For most studies, the maximum sorption was nearly or superior to 75%. The sorption was efficient at temperatures until 45 [degree]C and the presence of some salts did not affect significantly the efficiency of mercury removal. Based on these preliminary results it is possible to conclude that bixin incorporated on the PTFE filters is a promising material for recover Hg (II) and could be used in the future in filters for effluent purification or in sample preparation techniques to pre-concentrate the metal.
Bacterial Spores as Platforms for Multiplex Bioremediation

The use of spores as platforms for surface display of proteins presents advantages over cells due to the spore natural durability and resistance to harsh conditions. These features are also advantageous as compared to chemical immobilization of proteins on polymer beads or other supports, along with: (a) safe, simple, fast, cost-effective production of protein-displaying spores by biofermentation; (b) no need for time-consuming purification steps to separate proteins; (c) higher batch-to-batch reproducibility. Thus far, the use of spore surface display for analytical systems has been limited. In this presentation, we will discuss our work on surface display systems for bioremediation applications by the use of bacterial spore from \textit{Bacillus subtilis} as a novel platform for multiplex bioremediation. Specifically, we displayed the thermophilic enzyme L-2-Haloalkane Dehalogenase from \textit{Sulfolobus tokodai} (L-2-HADST) on the surface of \textit{Bacillus subtilis} by using its own spore coat proteins (CotA and CotB) fused to L-2-HADST and a C-terminal 6xHisTag. We initially compare CotA and CotB for their ability to surface-display L-2-HADST and afterwards focus on the CotA-L-2-HADST recombinant spores since they not only display the haloalkane dehalogenase on their surface, but additionally present laccase activity coming from spore Coat Protein A from \textit{Bacillus subtilis}. CotA can perform single-electron oxidation of a wide variety of aromatic compounds by using copper as a cofactor. Furthermore, we hypothesize that the high affinity of CotA for copper, together with a 6xHisTag fused on C-terminus of L-2-HADST, could be used for chelating of heavy metals. Altogether, the proposed spore-based technology has the potential to reduce the degree of toxicity caused by different groups of pollutants and provides with a novel bioremediation tool that can be tailored to the remediation of other classes of pollutants, having a beneficial impact on the environment.

Keywords: Biotechnology, Chemical, Environmental, Enzyme Assays
Application Code: Environmental
Methodology Code: Chemical Methods
Excess nutrients (phosphorus, nitrogen) in water leads to algal bloom, one of the most severe problems associated with surface water. Phosphorus is the limiting nutrient to control algal growth; therefore, removing phosphorus from surface water is a major strategy to prevent algal bloom in lakes and other water bodies. In this study, we present the development and evaluation of a NanoIron sponge as a phosphorus sorbent. Iron nanoparticles are grown directly on a polyurethane sponge using a proprietary technology involving thermal reduction. The results demonstrate that the NanoIron sponge can remove over 99% of phosphorus from water within 5 min. After passing the solution containing 10 mg L\(^{-1}\) phosphorus through the NanoIron sponge, phosphorus concentration dropped to the undetectable level (<2 µg L\(^{-1}\)), below the US-Environmental Protection Agency (US-EPA) limit. The NanoIron sponge has an uptake capacity of over 116 mg g\(^{-1}\), up to 4 times the capacity of commercially available phosphorus sorbents. The used sponge can be regenerated and 100% of phosphorus recovered by simple immersion in the regeneration solution. Furthermore, the sponge exhibits antibacterial properties against cyanobacteria, which prevents biofouling and thus allows a long-term use of the sponge in real-world conditions.

**Keywords:** Contamination, Environmental/Water, Isolation/Purification, Nanotechnology

**Application Code:** Environmental

**Methodology Code:** Chemical Methods
Environmental Combining High-Capacity Sorptive Extraction with Thermal Desorption Pre-Concentration for Analysis of VOCs and SVOCs in Environmental Samples

Analysis of volatile and semi-volatile organics in environmental samples often requires extensive sample preparation in order to efficiently extract and concentrate the target analytes prior to separation and detection. Thermal desorption has commonly been employed as a ‘solvent-free’ sampling approach for environmental air monitoring, but has historically been limited in its ability to deal with liquid matrices.

High-capacity sorptive extraction is a simple, fast sampling technique which may be employed to liquid samples, and coupled with TD for analysis by GCMS. Building on well established techniques such as SPME, this new approach can provide additional sample information due to its increased sample capacity, robust design, and compatibility with TD pre-concentration. This methodology can be applied to a range of environmental applications for S(VOCs) including water, wastewaster and soil samples. Polycyclic aromatic hydrocarbons (PAHs) are one example of a particularly harmful group of organic compounds which are of environmental importance. Analysis of these compounds often involves requires labor-intensive liquid extraction, which makes these methods difficult to automate. In this poster, we describe the development of High capacity sorptive extraction with TD–GC–MS method for a number of environmental samples including PAHs, using a new generation of thermal desorbers with outstanding capabilities for these challenging analytes.

Keywords: Environmental, Extraction, Semi-Volatiles, Thermal Desorption
Application Code: Environmental
Methodology Code: Gas Chromatography
Environmental Physicochemical and Toxicological Characterization of Engineered Nanoparticles and Chemical Additives Used in the Semiconductor Industry

Chemical mechanical planarization (CMP) is an essential step during the manufacturing process of integrated circuit chips in the semiconductor industry. During CMP, excess deposited material is removed from metallic substrates by chemical and mechanical means. The major component affecting the polishing process is the CMP slurry, an aqueous dispersion of engineered nanoparticles (ENPs) and chemical additives. The chemical additives selectively dissolve the materials present on the wafer surface, while nanoparticles, such as silica, ceria or alumina, mechanically remove the chemically modified surface through abrasion. None of these slurry components are incorporated into the semiconductor product, but they are eliminated through CMP effluents, along with dissolved and particulate materials removed by the polishing process. Therefore, it is essential to assess the environmental impact of the released nanoparticles and reactive additives. Our work is focused on studying the compositional change of CMP slurries throughout a particular CMP process. The interaction between nanoparticles and chemical additives during the polishing process is assessed. Zebrafish (Dario rerio) embryos are used as biological model to assess the toxicity of individual slurry components and their mixtures, before and after use in a typical CMP process. The mortality rate is used as a primary end-point. We observe that the interaction between nanoparticles, chemical additives, and removed materials affects the toxicological profile of released CMP waste. This work provides toxicological information of realistic CMP materials and can be used as a general guideline to design environmentally friendly slurries with predictive toxicological impact.

Keywords: Environmental Analysis, Materials Characterization, Nanotechnology
Application Code: Environmental
Methodology Code: Thermal Analysis
Quantitative Analysis of Inorganic Anions in Metropolitan and Bottled Water Samples Using Ultra-performance Liquid Chromatography/Electrospray Ionization Mass Spectrometry

Drinking water must be sufficiently safe for human consumption or should have a minimal threat of instantaneous or enduring harm [1]. Nitrate and nitrite are naturally occurring ions created by the oxidation of nitrogen in the presence of microbes. The United States Environmental Protection Agency (US EPA) and International Bottled Water Association have set the maximum contaminant levels for nitrate (10 mg L-1) and nitrite (1 mg L-1) in drinking water [2,3]. This high consumption revealed that bottled water is considered to be the safest source of drinking water in Saudi Arabia.

In the present study, a new, rapid and precise technique based on ultra-performance liquid chromatography/electrospray ionization mass spectrometry (UPLC-ESI/MS) has been optimized for the analysis of nitrate and nitrite in drinking water. The established technique was linear (R2 > 0.999) over the working concentration values, the run-to-run and day-to-day precisions were <4% (n = 5) in terms of relative standard deviation (RSD) when examining a nitrate and nitrite standard mixture of concentration 0.05 mg L-1, nitrate and nitrite detection limits were found to be 0.03 µg L-1 and 0.04 µg L-1, respectively. The proposed UPLC-ESI/MS technique has been employed effectively for determination of nitrate and nitrite in metropolitan and bottled water samples from the Saudi Arabia; ten metropolitan and twenty bottled water samples have been examined where nitrate and nitrite contents were obtained from 0.35 to 9.02 mg L-1.

Acknowledgment
The authors extend their appreciation to the Deanship of Scientific Research at King Saud University for funding the work through the research group project No. RG-1437-004.

References

Keywords: Chromatography, Environmental/Water, Liquid Chromatography, Liquid Chromatography/Mass Spec
Application Code: Environmental
Methodology Code: Liquid Chromatography/Mass Spectrometry
There has been an increasing awareness of the presence of PFAAs in water. A simple and robust method with quick turn-around time to determine these compounds is essential to provide accurate responses in a timely manner. Direct injection without SPE allow us to maximize throughput and to minimize background caused by the sample preparation step. We used Liquid Chromatography / triple quadrupole mass spectrometer to analyze the fluorotelomer and unsaturated fluorotelomer acids included in ASTM method 7968/7979. Fluorotelomer acids are observed as [M-H] - and [M-HF-H]-. Since the loss of HF in the fluorotelomer acids results in an ion with the same formula as the unsaturated fluorotelomer acids, and these two classes of compounds showed very similar retention times, we reduced ESI heater temperature to reduce HF loss and minimize false identifications. For method validation, we tested sample stability, effect of contaminant in mobile phase, etc. Calibration range and detection limit meet specified value. We also analyzed samples including tap water and surface water to validate optimized method.

**Keywords:** Environmental Analysis, Liquid Chromatography/Mass Spectroscopy

**Application Code:** Environmental

**Methodology Code:** Gas Chromatography/Mass Spectrometry
Environmental

The Most Cost Effective Green Solution for the Analysis of Volatile and Semi-Volatile Compounds in Air

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

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

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

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

Keywords: Air, Environmental/Air, Gas Chromatography/Mass Spectrometry, Thermal Desorption
Application Code: Environmental
Methodology Code: Gas Chromatography/Mass Spectrometry
Standard reference materials (SRMs) 1597a and 1991 are complex mixtures of polycyclic aromatic hydrocarbons (PAHs) derived from coal tar for which a selected number of PAHs have been assigned mass fraction values by the National Institute of Standard and Technology. The mass fraction values for these PAHs were assigned based on combining results from several methods including gas chromatography/mass spectrometry (GC/MS) and/or reversed-phase liquid chromatography with fluorescence detection (RPLC/FLD). The research presented here focused on the development of two new analytical methods for the separation and identification of MM 302 Da PAHs in SRM 1597a and SRM 1991. Due to the complexity of the sample matrix, the analytical methods described here require a fractionation step using normal-phase liquid chromatography (NPLC) on an aminopropyl (NH2) stationary phase. SRM samples were analyzed after fractionation by GC/MS using a 50% phenyl stationary phase and a RPLC/FLD on a polymeric C18 stationary phase with fluorescence detection. The NPLC-GC/MS and NPLC-RPLC/FLD methods presented here allowed for the identification of 21 MM 302 Da PAHs in SRM 1597a and SRM 1991.
Environmental

Extending the Analysis of Ozone Precursors – Continuous, Unattended, Cryogen-free On-line Monitoring of PAMS Hydrocarbons and Polar VOCs in Ambient Air by TD–GC–MS

The presence of volatile hydrocarbons in urban atmospheres is believed to contribute to the formation of ground-level ozone, one of the main constituents of urban smog. The compounds of interest range in volatility from acetylene to trimethylbenzene, and are generally referred to as ‘ozone precursors’. There is currently growing interest in carrying out continuous time-resolved measurement (‘on-line monitoring’) of ozone precursors and other compounds, for source apportionment and in order to better understand the factors that influence their concentrations. This is particularly the case in Asia, with recent strong growth in on-line monitoring of industrial emissions in China, following the mandating of a nationwide real-time air monitoring system in the 13th Five-Year Plan, and numerous regional controls on industrial VOC emissions.

The proposed list of target compounds for the re-engineered PAMS program comprises 28 ‘priority compounds’ and 35 ‘optional compounds’. However, it is widely recognised that numerous polar compounds (including oxygenated species such as alcohols, aldehydes, ketones and esters) and terpenoids play an important role in atmospheric chemistry, including in the formation of ozone and secondary organic aerosols.

As a result, there is a growing desire to ‘future-proof’ on-line GC systems for VOC monitoring, by ensuring that polar compounds as well as hydrocarbons can be monitored simultaneously. This has historically presented a challenge to analysts because of the tendency for volatile polar species and monoterpenes to be lost when conventional Nafion™ dryers are used to remove humidity from the air stream.

This paper describes an on-line thermal desorption (TD)–GC–MS system that overcomes this difficulty by using an innovative, cryogen-free water-removal device upstream of the thermal desorption focusing trap. The result is improved performance for an extended range of analytes that includes hydrocarbons, polar species and monoterpenes.

Abstract Text

Co-Authors: David Wevill, Ilaria Ferrante, Massimo Santoro, Mireia Aragon

Application Code: Environmental/Air, GC
Methodology Code: Sampling and Sample Preparation
Compared to the general population, firefighters are known to be exposed to hazardous chemicals, including, most notably, polycyclic aromatic hydrocarbons (PAHs) at higher concentrations than that of the general population. Furthermore, it is known that firefighters also have a comparatively increased incidence of certain cancer types, despite their protective turnout gear. Considering a probable link between exposure to PAHs and increased rates of cancer in firefighters, a resolution was made to devise a means for documenting the extent and mode of exposure. Our strategy involves the development of a chemically modified porous silicone-based wristband that is capable of trapping volatile organic compounds (VOCs). The wristband was pilot-tested as a personal sampling device for work environment exposure monitoring in active duty firefighters. The collected wristbands underwent multiple extraction steps, followed by GC-MS for final analysis to demonstrate the efficacy of modified silicone-based modalities in monitoring the types and quantity of VOCs in the firefighters’ surrounding environment with high reliability. Initial findings show multiple exposures to various PAHs and VOCs of concern for the health of the firefighters when in a fire environment. Among the PAHs found were Naphthalene, Acenaphthylene, Phenanthrene, and Pyrene. Current work with the wristbands is focusing on expanding the identification of the VOCs trapped in the wristbands worn by the firefighters when in active duty and assessing the impact of these environmental hazards to their health.
The Development of New Type of Cyclonic Spray Chamber for ICP-MS or ICP-OES Combining Both Advantages of Gas Based Sample Introduction and Coaxial Nebulizer

ICP-OES simultaneously quantifies concentrations of multielement simply and rapidly; thus, it can be widely used in the environmental and material field. In particular, heavy metal ions in ICP-OES analysis is significant in the administration of harmful metals due to keep various environmental regulations or laws. For instance, the administrative measurement of Cd, Pb, Cr, As, Se, Sb and Hg has been conducting in worldwide. However, in the point of sensitivity of ICP-MS, the sensitivity of As, Se, Sb, Hg in the direct ICP-OES measurement is relatively poor and it may be not sufficiently obtained. As a typical approach to overcome this problem, a hydride generation method (attachment) will be available. In contrast, if other elements (except above heavy metal ions) are measured, respective measurements are required by the use of versatile concentric nebulizer. In this study, new type of cyclonic spray chamber was fabricated combining both advantages of hydride generation attachment (or ultrasonic nebulizer) and concentric nebulizer. Namely, the proposed chamber has two sample-introduction ports both of the gas sample introduction port from hydride generation attachment (or ultrasonic nebulizer) and aqueous based sample from concentric nebulizer. In this presentation, the optimization of new chamber system and its application will be presented aimed at environmental analysis.

Keywords: Environmental/Water, ICP, ICP-MS, Ultratrace Analysis
Application Code: Environmental
Methodology Code: Atomic Spectroscopy/Elemental Analysis
Environmental Estimating Metal Burdens in Selected Freshwater Crayfish Species from Reference and Mining-impacted Ozark Streams (Southeast, Missouri)

Mining of metal ores such as Pb and Zn occurred for many decades in Southeast Missouri1. Mining is active at new sites and some old sites have been discontinued. Previous studies indicated ecological changes and elevated metal concentrations in the water column, sediment and the food chain1. Crayfishes are good bioindicators of heavy metal contamination in aquatic systems2. This study quantified As, Cd, Cu, Co, Fe, Ni, Pb, and Zn in the water column and in crayfish tissues (abdominal muscle, hepatopancreas, etc.) from reference and mining-impacted sites. Samples of water and the crayfish species were collected during four seasons (spring; summer; fall; winter) in 2016 from Mill Creek (reference site), and West Fork Black River (WFBR), and Brushy Creek (moderately-impacted sites). Crayfish species found in the streams sampled were the Belted Orconectes harrisoni, Freckled Cambarus maculatus, Golden O. luteus, Woodland O. hylas, and Spothand O. punctimanus). Water samples from the streams and the preferential distribution of metals in tissues of organisms were determined by inductively coupled plasma – mass spectrometry (ICP-MS). Organ-differentiated metal content in the species, the impact of mining activity, and seasonal variation on the metal body burden of the species will be presented.


Keywords: Environmental Analysis, Environmental/Biological Samples, Food Contaminants, ICP
Application Code: Environmental
Methodology Code: Atomic Spectroscopy/Elemental Analysis
Environmental
Investigating Lignocellulosic Biomass as Renewable, Non-Food Source of Biofuel and the Quest for an Efficient Pretreatment System

Barnabas Gikonyo
SUNY Geneseo

The incremental demand for energy in the World and the consequent rise in pollution levels from the use of fossil fuels has led to an ever increasing demand for alternative sources of fuel. A number of alternatives currently being employed require the use of food crops, which in turn has led to an increase in world food prices, more so in poor countries where a large part of the populace rely on these very food crops for sustenance. In 2008, the World produced well over 130 million tons of rice husks; with virtually all of it going to waste. Recent studies indicate that there is at least 50 million dry tons of sustainable woody biomass feedstock available for less than 40 dollars/ton! With 1 in 8 people suffering from chronic undernourishment in 2010-2012, and so much biomass available that can be converted into biofuel, the pursuit of the efficient, non-polluting, and recyclable pretreatment system begins in earnest. In this study, a series of ionic liquids (1-ethyl-3-methylimidazolium chloride, 1-butyl-3-methylimidazolium chloride, and 1-hexyl-3-methylimidazolium chloride) were used as pretreatment systems on rice husks, Douglas fir, alfalfa and crabgrass. The results are presented and discussed.

Keywords: Biofuels, Environmental, Environmental/Waste/Sludge, Fuels\Energy\Petrochemical
Application Code: Environmental
Methodology Code: UV/VIS
Environmental Airport Emission Rates from Airborne Measurements of NOx and CO2

The Los Angeles International Airport (LAX) emits gaseous pollutants and particulate matter that are harmful to the environment and human health. Each year since 2012, the NASA Student Airborne Research Program has conducted airborne missed approaches at LAX and other airports in the Los Angeles Basin, while making in-situ measurements of carbon dioxide (CO2) and the oxides of nitrogen (NOx) using instruments onboard the aircraft. The CO2 was measured via the NASA Goddard Greenhouse Gas suite, which uses infrared laser absorption spectroscopy combined with a multi-pass gas cell that gives an effective absorption path length of several kilometers and measurement precisions of less than 0.5%. The NOx was measured with a chemiluminescence-based instrument from the University of Virginia. We show that observationally derived NOx emission indices (grams of NOx emitted per kilogram of jet fuel consumed) from individual emission plumes sampled at low altitude along the LAX runway are consistent with the values for the expected aircraft engine type and mode that are listed in the ICAO Aircraft Engine Emissions Databank. Additionally, the vertical profiles from fifteen missed approaches were used to create a new model that can perform a top-down prediction of the total airport emission rate (tons of CO2 emitted per hour) for any airport with a known wind speed and frequency of aircraft departures. The measured top-down airport emission rates for LAX were compared to the standard bottom-up emission rates estimations using the NOx emission indices tabulated in the ICAO Aircraft Engine Emissions Databank. Finally, the top-down model was used to predict the airport emission rates for major airports throughout the United States.

Abstract Text
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Keywords: Chemiluminescence, Environmental/Air, Gasoline, Molecular Spectroscopy
Application Code: Environmental
Methodology Code: Molecular Spectroscopy
Water quality degradation including lead (Pb) leaching in drinking water distribution systems has been a national issue for the last decade. As a result, a great deal of research on understanding lead release mechanisms and developing corrosion control approaches has been undertaken. Although monitoring buffer water quality and forensic investigation such as metal surface characterization has also provided meaningful information, the dynamics of liquid-metal interfacial water chemistry, which is essential to understanding the corrosion processes, is still not well described due to the lack of experimental tools and techniques. This study utilized microelectrode technologies (tip diameters of 10–100 µm) capable of real-time in situ direct measurements of pH, dissolved oxygen (DO), free chlorine, and Pb concentrations changes near a fresh brass:lead galvanic coupon surface under different flow patterns and water conditions. Various one-dimensional (1D) concentration gradient microprofiles and 2D surface maps were constructed to evaluate the effect of different water qualities on pH, DO, free chlorine, and Pb concentrations at the metal surfaces. A total of 16 different drinking water conditions were evaluated using batch tests. Parameter profile measurements showed that, under stagnation with pH 7 and low alkalinity (e.g. 10 mg C L\textsuperscript{-1}), the difference between high and low surface pH was 7.5 between the cathodic brass (pH 10.3) and the anodic lead joint (pH 2.8), while the bulk water pH remained at 7.2. Free chlorine concentration at the surface showed much higher consumption under stagnation than flow condition. This work illustrated the value of microelectrodes for investigating liquid-metal interfacial chemistry change in a micro-environment of galvanic corrosion for better understanding of the related mechanisms, thus leading to effective corrosion control practice.

Keywords: Environmental/Water, Microelectrode, Sensors, Surface Analysis
Application Code: Environmental
Methodology Code: Sensors
In situ heavy metal detecting sensors can provide important, real-time field data for effective monitoring and managements of water systems such as groundwater. Among many available techniques, environmental monitoring systems using electrochemistry are considered to be complementary to the traditional techniques, promising inexpensive and portable instruments. Various electrochemical sensors have been developed based on metallic mercury (Hg), however, due to high toxicity of Hg, recent many attempts are to replace Hg to bismuth (Bi) as a heavy metal detecting material. In this study, we introduced an innovative nanoporous Bi electrode (NBE) design to improve electrode sensitivity, compatibility, and reproducibility for real-time Cd$^{2+}$ and Pb$^{2+}$ monitoring in groundwater. A novel electrochemically deposited Bi-Sn nanoporous film-modified carbon electrode was prepared through selective dealloying of Sn in sequence. Using square wave anodic stripping voltammetry (SWASV), the heavy metal stripping was conducted with 25 mV pulse amplitude, 20 Hz pulse frequency, and 4 mV square wave step voltage. Two distinguished peaks were observed at -0.65 V and -0.45 V, respectively, which are associated with the stripping of Cd$^{2+}$ and Pb$^{2+}$ in acetate buffer at pH 4.6. A sturdy Bi nanoporous structure increased surface to volume ratio and electron mobility for heavy metal detection, exhibiting longer lifetime (~2.7 times longer than a general bi-filmed electrode) with good stability (~40 repeated measurements). The newly developed Bi-Sn nanoporous sensor was successfully applied for detecting heavy metal ions in a simulated groundwater system and showed an excellent sensitivity and selectivity for measuring Cd$^{2+}$ and Pb$^{2+}$ with a good reproducibility. The limit of detection (LOD) was 5 µg L$^{-1}$ for both Cd$^{2+}$ and Pb$^{2+}$ ions.

Keywords: Environmental, Environmental/Water, Microelectrode, Sensors
Application Code: Environmental
Methodology Code: Sensors
The microbial world remains mostly unexplored as most bacterial species either do not grow in standard laboratory growth media or are outcompeted by a few well-known species. To address the current limitations of isolating and cultivating new and unique bacterial species, we have fabricated a microfluidic device that allows bacterial cells to isolate themselves and flourish in their natural environments so that they can be easily collected and analyzed.

We designed and fabricated PDMS microfluidic devices that contain sub-micrometer constrictions, which connect the external environment to individual isolation chambers. The isolation chambers allow nutrients to exchange with the environment. When placed into the environment, bacterial cells are driven toward the sub-micrometer constrictions that allow entry into the isolation chambers via positive or negative chemotaxis. Once a cell enters the constriction, the entrance is blocked and the isolated cell can replicate freely to populate the isolation chamber.

To demonstrate the technology, two microfluidic devices were placed in river water samples collected from the Charles River in Boston, Massachusetts. 11 unique bacterial species were cultured from only 14 isolation chambers. Using conventional plate culturing techniques, which require multiple dilution and sub-culturing steps, 22 bacterial species were eventually isolated. Interestingly, only 1 bacterial species was common between the two methods, emphasizing the diversity of bacterial species contained in a relatively mundane sample. This experiment highlights the reduction in manual effort that the microfluidic devices provide. We expect that the diversity of isolated species will continue to increase and quickly outpace conventional methods as additional microfluidic isolation chambers are deployed.

Keywords: Biological Samples, Environmental/Biological Samples
Application Code: Environmental
Methodology Code: Microfluidics/Lab-on-a-Chip
Electrochemical Investigation of Heavy Metal Ion Adsorption on Engineered Nanoparticles: Effect of Environmental Composition and Particle Surface

Metal oxide nanoparticles, especially titania (TiO2) and zinc oxide (ZnO) are heavily used in consumer products, notably in cosmetics and sunscreens and are discarded in the environment with little regulation. Although these nanoparticles are found to be relatively non-toxic, once in the environment they can undergo transformations and interact with small molecules and metal ions which drastically change their properties and toxicity profile. This presentation will discuss electrochemical studies to investigate the interaction of metal oxide nanoparticles with heavy metals in the environment. Examples of nanoparticle systems and studies to assess the effect of particle type, surface coatings and environmental composition will be provided along with the parameters controlling adsorption/desorption of toxicants, measured using electrochemical methods and a suite of spectroscopic procedures. We demonstrate the use of electrochemistry as a powerful tool to quantify heavy metal adsorption and determining mechanisms to predict the interaction of nanoparticles in the environment.

Keywords: Electrochemistry, Environmental, Nanotechnology
Application Code: Environmental
Methodology Code: Electrochemistry
This paper presents and discusses our continued studies of abandoned mine drainage (AMD) quality during and after processing at the Lowber, Pennsylvania passive treatment facility near the old Marchand coal mine. This study, in collaboration with the Sewickley Creek Watershed Association (SCWA), a Greensburg, PA-based natural resources conservation group, seeks to 1) assess selected analytes in AMD from the Lowber facility in the field and laboratory, and 2) provide assistance to the SCWA toward assessment of AMD and development of remediation strategies for use at potential sites throughout the Sewickley Creek Watershed. This poster presentation involves the continued research on AMD by one of our local high school student investigators. Thus far, she has contributed to our work by monitoring levels of alkalinity, acidity, sulfate, iron, and dissolved oxygen in mine drainage from the inlet pond, six settling ponds, wetlands, and outlet sections of the facility. In 2017-2018, she will determine calcium and sulfate concentrations, along with pH, dissolved oxygen, conductivity, iron, and alkalinity, throughout the Lowber facility and across the road at a sampling location in Sewickley Creek. The hypothesis is that calcium levels in AMD are suppressed by high (ca. 1000 mg/L) sulfate concentrations, and that calcium and sulfate levels in Sewickley Creek will be lower than those in the AMD from the Lowber settling ponds. Sample collection, preparation, analytical methods, and results obtained so far, and their significance to remediation of AMD, will be presented and discussed. Also to be presented and discussed are the benefits to our high school student researcher of involvement in this type of research experience, and future plans for this study overall.

**Keywords:** Environmental Analysis, Environmental/Water, Monitoring, Teaching/Education

**Application Code:** Environmental

**Methodology Code:** Education/Teaching
A novel approach to determination of iron, aluminum, and other selected metal ions of interest, using common chelating agents in aqueous as well as incorporated into an optically transparent membrane, followed by analysis of the data obtained via univariate and multivariate regression methods, e.g., partial least squares (PLS), to quantify the metals of interest, will be presented and discussed.

Applications of membranes to analyte detection have involved incorporation of the membrane into a sensor device for laboratory and field analyses, or as test “strips” for qualitative and semiquantitative analyses. Using membranes to detect metals involves incorporation of a metal-specific chelator plus necessary reagents in a polymeric gel. The goal of our investigation is to produce optically transparent agarose- and sodium alginate-based membranes impregnated with a chelating agent that ultimately will bind selected metal ions under proper conditions, permitting quantitation of the metal. Eriochrome Cyanine R, Chrome Azurol S, and Pyrocatechol Violet can chelate trivalent forms of aluminum and iron simultaneously in specific pH ranges, and are potential candidate chelators for individual and simultaneous analyte determination. Another chelator of interest for use with membranes is iron(II)-specific Ferene S, which will also be studied as part of this project.

Experiments will be conducted in solution and membrane media to test the feasibility of iron and aluminum determination. The Beer’s law behavior of each chelate of the candidate ligands will be investigated to define suitable experimental conditions. Simultaneous determination of trivalent iron and aluminum concentrations in selected sample types samples will be achieved by applying chemometric methods, e.g., PLS and least-squares minimization, to the spectrophotometric data obtained. The results obtained from our work will be presented and discussed, as will future plans for this research.
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<td>Abstract Title</td>
<td>Multiple Level Analytical Course-Embedded Pesticides Analysis for Water Samples</td>
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<td>Primary Author</td>
<td>Xiaoping Li, Georgia Gwinnett College</td>
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<td>Co-Author(s)</td>
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**Abstract Text**

This project involves a qualitative and quantitative analysis of one of the most widely applied pesticide (atrazine) in various real-world water samples, in both CHEM 3000K (analytical chemistry) and CHEM 4100K (instrumental chemistry) courses, with multiple separation/detection techniques. We have created a great opportunity to teach our students how to become better scientists through a problem-solving research project, and provide them hands-on experience in multiple instrumentations including Gas Chromatography-Mass Spectrometer (GC-MS), High-Performance Liquid Chromatography (HPLC), and Enzyme-linked Immunosorbent Assay (ELISA). This is a unique opportunity to demonstrate how to solve one practical problem through multiple approaches. The aims of the project are: (i) to solidify and to advance scientific knowledge and research methods through literature search and discussion, method development, and sample analysis. (ii) to promote logical and critical thinking skills through solving real problems, (iii) to have hands-on experience on modern instrumentations, and (iv) to improve the collaborative, writing and oral communication skills of our students.

**Keywords:** Environmental Analysis, Gas Chromatography/Mass Spectrometry, HPLC, Teaching/Education

**Application Code:** Environmental

**Methodology Code:** Education/Teaching
On-Line Chemical Reaction Monitoring Using a Low-Field Proton NMR System

We will describe our studies in developing an on-line system for reaction monitoring that is based upon the integration of Sequential Injection Analysis (SIA), a continuous flow technique which allows for automated sample pre-treatment, to a proton Nuclear Magnetic Resonance (NMR) spectrometer. The SIA-NMR approach improves the sensitivity and selectivity of the picoSpin 80 bench-top NMR spectrometer by effectively pre-concentrating the sample as well as removing matrix interferences before the sample zone enters the flow cell of the spectrometer. We have studied the Fischer esterification reaction and the Maillard reaction, which is a complex series of reactions that occur when an amino acid condenses with a carbohydrate, with implications in environmental chemistry, food chemistry, and biochemistry. The initial steps of the Maillard reaction were modeled by using [alpha]-dicarbonyl compounds (e.g., glyoxal and methylglyoxal) and primary amines (e.g., phenylalanine-methylester). This approach allowed not only for a better understanding of the initial stages of the Maillard reaction but also of the complex solution chemistry of [alpha]-dicarboxyls. Results will be presented for our examination of several SIA sample pre-treatment methods, including approaches based upon solid-phase extraction, membrane dialysis, and liquid-liquid extraction. Integration of the SIA to the NMR for on-line reaction monitoring, with cycle times ranging from 5 to 30 minutes, will be described. This approach allows for the determination of rate constants, reaction orders, and elucidation of the structures of the intermediates formed. Kinetic studies using the SIA-NMR system forming the basis of an experiment for the undergraduate curriculum in analytical chemistry will also be presented. This work was supported by the Research Growth Initiative & the Office of Undergraduate Research at UW-Milwaukee and by a grant from Thermo Fisher Scientific, Inc.

Keywords: Environmental/Soils, Flow Injection Analysis, NMR, Teaching/Education

Application Code: Environmental

Methodology Code: Magnetic Resonance
Toxic organic compounds (TOCs) are monitored in various places (i.e. workspace air, ambient air). The most representative analysis method of TOCs is U.S. EPA Compendium Method TO-17. In this method, collecting volatile organic compounds (VOCs) in sorbent tubes using active sampling pump and analyze them with Thermal Desorption-Gas Chromatograph/Mass Spectrometer (TD-GC/MS), however, which takes time to collect sample. So it is common to collect samples in multiple tubes or use TD restore function.

With the restore function, VOCs in sorbent tube are desorbed and trapped on 2nd trap, then 2nd trap is heated and VOCs are introduced into column with split injection mode. While injecting, split flow are once again trapped into the sorbent tube. Even if a problem occurs, the sample can be measured again, so that even precious trace samples can be analyzed without difficulty. The problem of restore function is difficulty of trapping wide range compounds from low-boiling compounds to high-boiling compounds at the same time. When restoring the sample, it is difficult to trap low-boiling compounds because the sorbent tube remains high temperature just after the desorption. It is common to replace the sorbent tube before injection, however the risk is of replacing sorbent tube is leakage. If leakage occurs, both analysis and restore are failed. To avoid failure, restoring original sorbent tube is effective. It is necessary to cool the sorbent tube quickly, and set the temperature of sample line as low as possible. Besides, if the temperature of sample line is too low, high-boiling compounds likely to carry over.

In this study, we consider the analysis condition of TD-GC/MS restore function to collect wide boiling range compounds with an excellent recovery factor. In poster presentation, we will report the analysis result of STD and real samples following U.S. EPA Compendium Method TO-17 to evaluate the sensitivity and quantitativity using restore function.
Environmental Photochemical and Photocatalytic Degradation of 1-Propanol Investigated by CE-C4D

Mauro S. Santos  
University of São Paulo

Carlos D. Garcia, Ivano G. R. Gutz, Thiago Gomes Cordeiro

Abstract Text

1-propanol is a primary alcohol mainly used in the pharmaceutical, chemical, and food industries. It has been found as a contaminant in the atmosphere and in wastewater. The UV degradation of 1-propanol has been investigated in the gas phase and propanaldehyde (main product), propionic acid, acetaldehyde and acetic acid were formed [1]. In this work, the formation of carboxylic during the photodegradation of 1-propanol in aqueous phase was measured by CE-C4D and the influence of UV exposure time, H2O2 and TiO2 was evaluated.

Solutions of 1-propanol (1mM) contained in quartz or plastic cuvettes were placed in a carousel stand 10cm apart from the quartz bulb of a 300W high pressure UV mercury lamp inside an air cooled photoreactor. The effect of the addition of a photocatalyst and/or an oxidant, respectively TiO2 (50–750mg/L) and H2O2 (0.05–2.00% v/v), was evaluated as well as the irradiation time (1–60min). All CE experiments were carried out using a silica capillary of 50µm i.d. and 55cm length (10cm effective). Injection time was 10s under 5kPa and the separation voltage was 30kV. The BGE used was 30mM CHES and 15mM NaOH (pH 9.2).

A greater amount of carboxylic acids was formed in quartz than in plastic cuvette (not transparent at WL<320nm) when 1-propanol was irradiated, even in presence of TiO2, that promotes the photocatalytic route, indicating a significant contribution of concomitant photochemical process under UV-C radiation. Addition of H2O2 (0.1%) tripled the generation of carboxylic acids. While propionate and formate peaks increased with the H2O2 concentration, an exponential decrease was observed for malonate and acetate (Fig. A). Regarding the effect of the irradiation time, the yield of propionate and formate increased up to 10min and of malonate and acetate up to 30min, remaining constant or decreasing afterwards (Fig. B).

Acknowledgments
To CNPq (Brazil) for fellowships and grant.

References

Keywords: Capillary Electrophoresis, Environmental/Water, Semiconductor

Application Code: Environmental

Methodology Code: Capillary Electrophoresis
Environmental Automation of EPA Method 528 - Determination of Phenols in Drinking Water

EPA Method 528 is used to determine phenolic compounds in raw and finished drinking waters. One (1) liter of dechlorinated and pH adjusted water samples are passed through polystyrene divinylbenzene (SDVB) copolymer solid phase extraction (SPE) cartridges. The analytes of interest are recovered with a dichloromethane bottle wash which is used as elution solvent for analyte recovery from the dried sorbent bed. Any residual water is removed with subsequent sodium sulfate drying of the elution solvent prior to concentration and analysis by gas chromatography/mass spectroscopy (GC/MS).

The PrepLinc\textsuperscript{TM} Large-Volume Injection (LVi) system allows the analyst to pass large volumes of aqueous sample matrix through commercially available SPE cartridges and disks. The autosampler will accommodate 1 liter sample jars, therefore sample is taken directly from the container used to collect the water in the field. The sample jar rinse is used to recover the analytes of interest from the SPE stationary phase directly to collection tubes, or to an AccuVap\textsuperscript{TM} module for concentration directly to autosampler vials. In this study, the labor-intensive steps of SPE conditioning, loading, elution and concentration were reduced to two steps: 1) loading samples on the PrepLinc\textsuperscript{TM} LVi system, and 2) programming the sequence with saved method parameters. This provided for a fast and simple, automated method for the labor intensive process of manually loading 1 liter of water to an SPE, subsequent elution and concentration for analysis. An evaluation of analyte recovery using several commercially available SDVB cartridges is presented.

Keywords: Environmental/Water, Sample Preparation, Solid Phase Extraction, Water
Application Code: Environmental
Methodology Code: Sampling and Sample Preparation
Pharmaceutical and personal care products (PPCPs) are introduced into our environment through numerous pathways including human disposal and animal runoff. Many of these PPCPs pose levels of environmental risk not fully accessed or explored by the current literature. One class of pharmaceutical products that requires significant work due to its implications on human health is antibiotics. The presence of antibiotics in the environment leads to antibiotic resistant bacteria, which are capable of producing infections in humans. In order to better understand the magnitude and severity of this emerging issue, antibiotic levels were monitored at wastewater treatment plants in southern Illinois in the influent, effluent, and effluent-affected streams. Aqueous samples were analyzed using filtration and solid phase extraction, followed by liquid chromatography mass spectrometry. Additionally, toxicological studies were performed to investigate the relationship between antibiotic concentration and antibiotic resistance conferral using bacterial cultures. Finally, antibiotic accumulation was investigated using model aquatic organisms to determine the concentrations and location of organismal accumulation of the antibiotics. The concentration levels were determined using LC-MS, while the location data was determined using imaging approaches. It was found that there are significant levels of antibiotics in both the influent and effluent, with only moderate levels of removal for most antibiotic species. This leads to easily detectable levels in effluent-affected streams that may produce deleterious effects over long-term exposure. Short-term exposure modeling demonstrated that significant levels of antibiotics accumulate in aquatic organisms.

**Keywords:** Environmental/Biological Samples, Environmental/Waste/Sludge, Liquid Chromatography/Mass Spec

**Application Code:** Environmental

**Methodology Code:** Liquid Chromatography/Mass Spectrometry
Artificial water-treatment wetlands are designed to reduce nitrogen and phosphorous nutrient concentrations in wastewater effluent to improve water quality and decrease eutrophication in natural waters. The Orlando Easterly Wetlands (OEW) is an engineered wetlands that polishes wastewater, lowering the total nitrogen from 2.31 mg/L to 1.0 mg/L, and phosphorous from 0.13 mg/L to 0.03 mg/L through biological, sedimentary, and chemical processes. Additionally, the wetlands provide habitat for avian, mammalian, and reptilian species. Previous research demonstrates that birds affect the eutrophication of agricultural reservoirs near their roost. The current research seeks to use uric acid (UA), an avian and reptilian excretory product, as a measure of the non-anthropogenic contribution to nitrogen within the park. Enzymatic fluorimetric analysis was utilized with two enzymatic reactions: uricase catalyzed reaction of UA to allantoin with production of hydrogen peroxide, followed by horseradish peroxidase catalyzed reaction of hydrogen peroxide with Amplex Red to form the fluorescent product, resorufin. Fluorimetric analysis was conducted (ex=530 nm, em=590 nm) with emission intensity proportional to UA concentration. UA was quantified throughout the OEW flow train, as well as an analysis of avian, alligator, and fish excreta to determine UA additions to the wetlands. High concentrations of ~10 M UA are found at the inflow and similarly high concentrations through deep marsh regions, but decline drastically through mixed marsh and outfall water. This information will help inform park personnel of water polishing efficacy and may prompt prescriptive changes to aquatic vegetation.
Environmental Stable CNT-based Printed Thin Film Multi-Sensor System

Carbon nanomaterial– and carbon nanotube (CNT)–based electronics continue to be an area of great interest due to their exceptional chemical and physical properties. Advances in printing technologies have aided the use of carbon nanotube–based fabric or matrix structures in electronics for the synthesis of large-area devices on flexible substrates. Over the past ten years, Brewer Science has been actively working on CNT-based fabric or matrix-based printed electronic devices starting from the fundamental theory of advanced CNT materials all the way to design and development of CNT device, sensor, and system products.

This presentation will demonstrate an example of this advanced printed electronic technology in the form of a four-pixel micro-array device featuring individual pixel temperature control for applications in environmental chemical and biological analysis. This pixelated array is made from a chemically doped CNT ink coated on a polyimide substrate. The pixels are temperature controlled between 60 and 180 degrees C using a unique pulse-heated electronic control system. The CNT fabric or matrix has the unique property that it can maintain a minimum of structural damage with current densities of as high as 5000 A/cm2. The printed electronics production process utilizes a customized approach including purification of raw CNT materials, ink formulation and doping, high-resolution and high-quality print processes, unique device architectures, and cost-efficient manufacturing. The multi-pixel temperature-controlled sensor system provides a low-power, accurate, stable, and reliable platform to perform thermal chemical analysis including environmental gas and volatile detection and measurement. The platform can also be used to thermally cycle through chemical activation processes for various detection applications. Detailed information and technical data will be presented at the conference.

Keywords: Detection, Environmental, Nanotechnology, Sensors
Application Code: Environmental
Methodology Code: Sensors
Environmental Modeling Transformation of Atmospheric CO$_2$ to Microalgal Biomass

Due to increased industrialization, the atmospheric CO$_2$ concentration continues to rise which is considered as one source of global warming. On the other hand, ubiquitous marine microalgae counterbalance this by sequestering this greenhouse gas via photosynthesis. It has also been found that the cells’ chemical environment has a major impact on the production of microalgal biomass. In order to quantitatively predict CO$_2$ sequestration and microalgal biomass production, it is crucial to accurately describe the relation between nutrient concentration and resulting biomass. However, since cells can only chemically interact with their microscopic vicinity, compound sequestration must be investigated on a micrometer spatial resolution. However, macroscopic ecosystems cannot straightforwardly be studied experimentally on microscopic scales especially when cultures contain large number of cells. To overcome this limitation, modeling methodologies will be presented which describe sequestration processes within microscopic vicinities of a large number of cells. These innovative modeling approaches are based on describing CO$_2$ dissolution, its transport to consumers as well as species-specific compound uptake and utilization. Moreover, real-world ecosystems contain multiple microalgae species that compete for a common nutrient source. Hence, competition effects among species have been incorporated into these novel models, too. Model validation has been accomplished under series of chemical conditions by comparing the predicted cell concentration with flow cytometry experiments. Very good agreement between model and experiment has been found in single-species cultures as well as for multiple species in a competition situation.

Keywords: Environmental Analysis, Method Development, Quantitative
Application Code: Environmental
Methodology Code: Computers, Modeling and Simulation
Multiple studies have been done to show the effect of unused drugs, fertilizer runoff and contaminants on the environment. The chemicals end up in plants consumed by animals and humans, and in beef, pork, chicken and fish. Detection of these chemicals continually challenged analytical scientists to identify and use the best analytical methods and protocols to optimize quality and efficiency in their work. Over the past few years, CAS has been working to develop a curated compilation in a variety of application areas including mass spectrometry. MethodsNow[registered], provides access to methods for detection of many of environmental contaminants. Methods are templated and characterized. Templates contain information on materials and instruments, sample and standards preparations, many additional experimental details and validation data. We will present case studies to illustrate how MethodsNow can be used to find experimental details for detection of contaminants in a variety of sources using any number of mass spectrometry techniques.

Keywords: Environmental Analysis, Gas Chromatography/Mass Spectrometry, GC-MS, Software
Application Code: Environmental
Methodology Code: Gas Chromatography/Mass Spectrometry
Consumption of cannabis and/or cannabis based products is currently legal in some form in 28 US states plus the District of Columbia. Testing of the plant materials and products is required by many of these states; however the specific test methods and target compound lists are not mandated in all cases. In October of 2016, the state of Oregon took a major step forward by requiring that all labs testing cannabis be accredited by the Oregon Environmental Laboratory Accreditation Program (ORLEP) and licensed by the Oregon Liquor Control Commission (OLCC). Consequently, Oregon Administrative Rules (OAR) list specific contaminants to be tested in marijuana samples, along with action levels. The pesticides on this list include carbamate, organophosphorus, macrocyclic lactone, neonicotinoid, pyrethroid, and triazole fungicides as well as others. A common approach for determination of these residues is the use of QuEChERS extraction followed by both LC/MS/MS and GC/MS/MS analysis. The extracts produced by this method present several challenges to chromatographic analysis; such as background from co-extracted matrix components such as chlorophyll and cannabinoids, and peak shape issues in LC. In this presentation, we will show a specific approach to address these issues in the analysis of cannabis samples spiked with pesticides included on the OAR list. Aspects will include column and mobile phase selection for LC/MS/MS, and the use a new cleanup sorbent blend for QuEChERS that offers several advantages over PSA/C18/GCB.
Molecularly Imprinted Sensors for Determination of Pesticides and Chemical Threat Agent Simulants

Molecular recognition in molecularly imprinted polymers (MIPs) is attributed to complementary binding sites with same or similar size, shape, and functionality to imprint molecules. A new selective and sensitive sensor based on molecularly imprinted poly(vinylidene fluoride) (PVDF) polymer sensor for the detection of parathion methyl (MP), a ubiquitous highly hydrophobic pesticide that is commonly used as a simulant of chemical threat agents. The PVDF sensor was prepared using the molecular imprinting method with a pre-polymerized PVDF instead of traditional in-situ or post-polymerized ones to avoid harsh polymerization conditions and simplify preparation process. In addition to size and shape of the imprinted cavities, the developed PVDF sensors could exhibit high selectivity and sensitivity mainly due to dipole-dipole interaction, hydrophobic interaction and van der Waals interactions with the template MP molecules. The results showed that the prepared PVDF sensor indeed showed high selectivity towards MP against other pesticides such as diethyl phosphoramidate, dicrotophos, 2,4,5-trichlorophenoxyacetic acid, secbumeton, and terephthalic acid achieving a limit of detection (LOD) of 68.0 nM and a limit of quantitation (LOQ) of 226.8 nM using quartz crystal microbalance as sensor platform. The specificity and selectivity of the prepared MIP were further verified with MIP-based solid phase extraction (SPE) and detection of the analyte in spiked vegetable samples.

Keywords: Biosensors, Environmental, Environmental/Water, Sensors
Application Code: Environmental
Methodology Code: Sensors
Recently, chemosensors based on polymer conjugated metallic nanoparticles (MNPs) have attracted great attention due to several important advantages, such as simplicity, signal amplification, easy fabrication, and different outputs, etc. Moreover, conjugated polymer chains with multiple recognition elements can increase both the binding efficiency and recognition selectivity for specific analytes. In addition, incorporating different MNPs into a functional polymer can produce a valuable conclusion based on the enhancement of outputs. Here, we selected poly(2-vinylpyridine)(P2VP) based amphiphilic block copolymers as the functional polymers owing to their excellent chelating ability due to presence of lone pairs of electrons on nitrogen atoms, and good film forming ability.

In this study, P2VP based block copolymers, poly(2-vinylpyridine)-block-polystyrene (P2VP-b-PS) and poly(2-vinylpyridine)-block-poly(methyl methacrylate) (P2VP-b-PMMA) have been utilized for the stabilization of metallic nanoparticles such as gold and silver. Further, these polymer conjugated metallic nanoparticles have been applied for applications as chemo-sensors. The P2VP-b-PS conjugated AgNPs based optical sensor is used for the detection of pesticides. The sensor was found to be selective for cartap, an analogue or precursor for natural insecticide Nereistoxin, Moreover, the detection system is consistent in the presence of many interfering pesticides and ions in the real samples. The optimized P(S-VP)-AgNPs based quantitative assay would potentially lead to more practical applications because of its low cost, simple preparation, excellent selectivity, and low detection limit.

Furthermore, P2VP-b-PMMA conjugated AuNPs proves to be an efficient and specific electrochemical sensor for nicotine, an alkaloid and a potent parasympathomimetic stimulant, in various tobacco containing samples (i.e. cigarettes) both in aqueous and organic media with fairly low detection limit.

**Keywords:** Biological Samples, Environmental/Water, Nanotechnology, Sensors

**Application Code:** Environmental

**Methodology Code:** Sensors
Water programs often require analysis of total nitrogen. EPA regulations require 40 CFR Part 136 approved methods for NPDES permits. Currently, total nitrogen is determined by adding the results of analysis of TKN and nitrate plus nitrite. This presentation describes a new ASTM method that determines total nitrogen in a single analysis using a TOC analyzer with chemiluminescence detection. How the method works, comparison with TKN plus nitrate nitrite, and results of a collaborative study will be presented.

**Keywords:** Environmental, Environmental/Water, Total Organic Carbon, Wet Chemical Methods
Hydrogen sulfide (H2S) and other sulfur compounds are invariably found in fossil fuels, natural gas and also in municipal sewage. H2S is highly toxic and corrosive required to be removed to make environment benign. Various materials such as zeolites, metal oxides and activated carbon have been studied for the removal of H2S. In this work, an efficient catalyst has been devised adopting new synthetic strategy whereby ZnO decorated MWCNTs has been successfully employed for the selective removal of H2S.

We demonstrate solvent-less in situ sulfidation of ZnO on the surface of functionalized MWCNTs carried out at ambient temperature using 10% H2S in N2 atmosphere. The resultant ZnS-decorated MWCNT is investigated by various spectroscopic and electron microscopic techniques indicating facile conversion of ZnO to ZnS on the surface of MWCNTs. The transformation of ZnO-decorated MWCNTs to ZnS-decorated MWCNTs was investigated by XRD. The EDX shows strong presence of S along with C and Zn peaks which confirms the existence of ZnS with MWCNTs. Further, presence of S2p peak in XPS analysis at ~ 162 eV confirms the formation of ZnS-decorated MWCNTs on sulfidation. Morphological and structural characterization of MWCNTs, ZnO-decorated MWCNTs and ZnS-decorated MWCNTs were investigated by TEM. Significant particle aggregation was seen at multiple regions on MWCNTs surface on sulfidation process whereas ZnO was fairly dispersed on the surface of MWCNTs. Phase identification was further complemented by SAED. H2S adsorption studies indicate that ZnO-decorated MWCNTs (80mg H2S adsorbed/g adsorbent) offer higher H2S adsorption than pure ZnO (11mg H2S adsorbed/g adsorbent). Thus, solvent-free H2S–mediated sulfidation method employing ZnO decorated MWCNT might be potential candidate in oil industry to curb H2S slippage.
**Environmental Analysis of Greenhouse Gases in Ambient Air by GC/FUV**

On January 1, 2010, the U.S. Environmental Protection Agency required large emitters of heat-trapping emissions to begin collecting greenhouse gas data under a new reporting system. Nitrous oxide, carbon dioxide, and methane are considered the main greenhouse gases in the earth's atmosphere. These gases absorb strongly in the infrared trapping heat in the atmosphere and affecting the temperature on the earth. These gases have been measured using expensive long path infrared analyzers or complex gas chromatography using as many as three detectors and three valves. We developed a far UV absorbance detector that we will apply to analysis of all three gases. It will just require a concentrator to detect ppb levels of these gases. The FUV detector recently has been improved so that it can detect low ppm levels of these compounds. We had developed a concentrator for nitrous oxide that could also be used for carbon dioxide and plan some additional changes to the FUV to improve its sensitivity to methane.

**Abstract Text**

On January 1, 2010, the U.S. Environmental Protection Agency required large emitters of heat-trapping emissions to begin collecting greenhouse gas data under a new reporting system. Nitrous oxide, carbon dioxide, and methane are considered the main greenhouse gases in the earth's atmosphere. These gases absorb strongly in the infrared trapping heat in the atmosphere and affecting the temperature on the earth. These gases have been measured using expensive long path infrared analyzers or complex gas chromatography using as many as three detectors and three valves. We developed a far UV absorbance detector that we will apply to analysis of all three gases. It will just require a concentrator to detect ppb levels of these gases. The FUV detector recently has been improved so that it can detect low ppm levels of these compounds. We had developed a concentrator for nitrous oxide that could also be used for carbon dioxide and plan some additional changes to the FUV to improve its sensitivity to methane.

**Keywords:** Environmental/Air, Gas Chromatography, Portable Instruments, Separation Sciences

**Application Code:** Environmental

**Methodology Code:** Gas Chromatography
A reliable method for the analysis of mercury species is cold vapour generation of mercury coupled with atomic fluorescence spectroscopy. The most commonly used method of cold vapour generation involves oxidising all organic mercury species to inorganic mercury (Hg2+), followed by a reduction to elemental mercury (Hg0) with tin(II) chloride or sodium borohydride. However, this method uses a plethora of chemicals which is both costly and prone to error as well as instrumental issues due to the complex wet chemistry necessary.

Another pathway is photochemical vapour generation (PVG), which has been used in the past in conjunction with a variety of detection methods such as atomic fluorescence spectroscopy (AFS) as an alternative method of cold vapour generation. PVG has been applied to total mercury measurements using UV with formic acid and acetic acid. In addition, the total mercury concentration in white vinegar was determined by matrix assisted photochemical vapour generation. PVG promises a much simpler and more cost effective approach, which uses fewer and more environmentally friendly chemicals to give results which are comparable or even more sensitive than the chemical vapour generation approach. Here we apply acetic acid photochemical vapour generation of mercury to speciation measurements to provide a much simpler analysis of methylmercury in four selected matrices: seafood, hair, sediment, and rice. In total, 9 certified reference materials were analysed to assess the accuracy and precision of the method. A comparison was made for the methylmercury signal obtained between the PVG and CVG methods, which showed that the PVG method provided a sensitivity equal to that of the CVG method.
Cyanobacteria in water sources could cause serious health risks to the public especially after entering the drinking water treatment plants. *Cylindrospermopsis raciborskii* is one of the most abundant and common species responsible for hazardous algal bloom. In this study, various oxidants were applied to treat the *Cylindrospermopsis raciborskii* in different source waters to investigate the release and removal of cylindrospermopsin (CYN). An ultra-fast liquid chromatography tandem mass spectrometry (UFLC-MS/MS) method was optimized and validated to monitor cyanotoxin concentrations. One of the objectives for a collaborative proactive study with the City of Tulsa Water and Sewer Department is to develop guidance for control and removal of cyanobacteria (*Cylindrospermopsis raciborskii*) and related cyanotoxins, if these types of algae bloom happen in the drinking water system, for Tulsa drinking water treatment facilities. Various dosages of free chlorine, chlorine dioxide, permanganate, and peracetic acid were added to Tulsa’s raw water at different reaction times, intracellular and extracellular CYN were monitored. At the same time, the removal efficiency of algaecides EarthTec® and copper sulfate on these cyanobacteria and algal toxin were also evaluated. The detailed experiment conditions, procedures, and results will be presented at the conference. This research is supported by Tulsa Metropolitan Utility Authority.
Gas chromatography coupled with a low-resolution mass spectrometer is a powerful analytical technique for identifying, confirming and quantifying organic compounds in complex matrices. Electron ionization (EI) produces a fragmentation pattern that is generally considered instrument independent. This has led to availability of a large database of EI library spectra like the NIST/EPA/NIH mass spectral library that contains hundreds of thousands of compounds and is primarily used for identification of “known” unknown compounds. Determination of unknown compounds by library matching has traditionally followed a ‘Chromatography-first’ approach. ‘Chromatography-first’ approach involves peak detection by one of the several available algorithms (like Genesis, ICIS, COBRA, etc.) and matching the detected peaks with the library to determine the closest matching compound. One of the flaws of using this approach to determine unknown compounds is that co-eluting compounds can be difficult (or almost impossible at low-levels) to identify. The deconvolution software takes the ‘Mass spectrum-first’ approach. This approach identifies compounds that would be co-eluting chromatographically but whose mass spectrum peak apexes are still separated.

In this study we revisit the concept of deconvolution and show its utility in identifying unknown compounds at both high and low level concentrations. We show how “mass spectrum-first” approach has assisted in identification of pesticides in complex matrix with the ability to deconvolve not only coeluting pesticides at high levels, but also the ability to identify low level pesticides that are essentially “buried” in the noise of the matrix. With the availability of advanced filtering capability and retention indexing feature we hope this new approach of looking at GC/MS data will significantly decrease the burden of high-throughput labs that are screening for specific class of compounds like pesticides or drugs of abuse.

Keywords: Data Analysis, Gas Chromatography/Mass Spectrometry, GC-MS, Pesticides
Application Code: Food Safety
Methodology Code: Gas Chromatography/Mass Spectrometry
Stir-bar sorptive extraction (SBSE) has been proven to be an effective and green extraction method for persistent organic pollutants (POPs) in aqueous samples such as human blood, drinking water, and wastewater. In this study, SBSE was used as an extraction method for analysis of POPs in dietary supplements. The extraction was followed by automated thermal desorption and GC triple quad MS. Compared with GC single quad, GC triple quad was able to provide higher sensitivity due to its capacity of multiple reaction monitoring. The quantification was achieved using isotope dilution mass spectrometry (IDMS) and compared with the results using calibration curve. IDMS relies on enriched isotopes spiked in the sample matrix. By measuring the isotope ratio using mass spectrometry, the concentration of analytes in the sample is calculated. IDMS can eliminate the error from recovery and provide accurate and precise quantitative results without using calibration curve. This novel extraction and quantification method was optimized and applied to the analysis of POPs in dietary supplement samples. Multiple polycyclic aromatic hydrocarbons and organochlorine pesticides were detected in some dietary supplements that are commercially available on the US market. This method was also adapted and applied to analysis of POPs in human blood. The target POPs included pesticides and industrial chemicals which were recently found in children’s blood and the environment. Bovine blood was used for method optimization and validation. Development of this accurate, precise, and sensitive quantification method is important for exposomic research.

Keywords: Food Contaminants, GC-MS, Isotope Ratio MS, Tandem Mass Spec
Application Code: Food Safety
Methodology Code: Gas Chromatography/Mass Spectrometry
The dietary value of edible seaweeds has prompted their consumption as a healthy food worldwide. Nevertheless, the detection and regulation for the hazardous residues in edible seaweeds are not fully established, making their analysis more meaningful and interesting.

Liquid-liquid extraction (LLE), a time-consuming and laborious technique, requiring large amounts of toxic organic solvents, is the accepted method for determination of residues in seaweeds. Solid-phase microextraction (SPME), instead, integrates sampling, extraction, concentration and sample introduction into a single, low-solvent consuming and automatable step, and it has been used for analysis of organic contaminants in different food matrices. Very few reports mention the use of SPME for seaweeds analysis, especially for poorly volatile compounds, such as PAHs, PCBs and pesticides.

This method is the first report of the use of matrix compatible PDMS/DVB/PDMS coating for the direct immersion (DI)-SPME of multiresidues in dry seaweeds and the simultaneous quantitation of contaminants belonging to three different chemical classes. Due to the wide range of polarities of the targeted contaminants, two DI-SPME protocols were optimized by multivariate experimental design strategies to evaluate all targets and a specific group of hydrophobic compounds, respectively. The best comprised conditions suitable for all targeted compounds, allowed the achievement of limits of quantitation (LOQ) for most compounds in the range of 1-20 µg kg⁻¹, a wide linear range of 5-2000 µg kg⁻¹ as well great correlation coefficient (R² >0.99) without significant lack of fit (p>0.05) at 5% level and satisfactory accuracy and precision values. Under the optimized conditions for hydrophobic analytes, lower LOQs were obtained ranging from 0.18-7.8 µg kg⁻¹. The established method was used for the screening of commercial dry seaweeds, and PCBs and PAHs were detected in some samples.

**Keywords:** Food Contaminants, GC-MS, SPME, Statistical Data Analysis

**Application Code:** Food Safety

**Methodology Code:** Gas Chromatography/Mass Spectrometry
Some ingredients in beer can be challenging to separate and chromatograph. Common beer flavor components include; fusel oils, aldehydes, esters, and organic acids. The polarity of a 6% cyano propyl phenyl stationary phase (624) has been a traditional choice for this type of analysis and works well. However, peaks shapes and low level detection of organic acids have been problematic for this phase. When inertness performance verified 624 columns are used, consistent organic acid performance is achieved. Static headspace GC/MS is an excellent way to profile alcoholic beverages such as a centuries old favorite malted barley beverage, beer. The recipes and components in these beverages are as diverse as the individual yeast strains and varietal hops that are on the list of ingredients. What flavor components are positive indicators of a good beer and which are not is one facet of the craft of making fine beer. Single Quad GC/MS is a great starting point for helping to indentify and monitor flavor components. Static headspace GC/MS chromatograms of beers and spirits illustrate the value of using inertness performance verified 624 columns for this type of analysis. Key elements of this type of analysis are the separation of amyl and iso-amyl alcohols, their esters, aldehyde peak shapes and detector response for organic acids. Obtaining headspace profiles can be a tremendous aide in understanding flavor development, tracking flavor stability and authentication of fine crafted alcoholic beverages. Comparing GC/MS profiles of various summer style beers and some of their flavor elements will keep things hopping.
Natural products commonly have subtle differences. The Beverage Industry may add supplements to enhance taste, extend shelf life, or modify aspects of the product. Frontier has developed a simple solid phase extraction technique to concentrate organic compounds from gaseous and liquid matrices. A chemically bonded PDMS layer on a titanium tube extracts the organic compounds. Tube is placed in deactivated stainless steel cup, desorbed in the pyrolyzer, and vapors Cryo-focused in a narrow band at the head of an analytical column. A chromatographic run commences utilizing both MS and the selectivity of the NPD simplifying detection for compounds of interest.
The fruit quality (FRUITY) project aims to provide a better understanding of post-harvest storage conditions of fruit, to allow improved sensorial and internal quality of fruit throughout the supply chain.

The project uses a multi-trait approach - including sensory profiling, monitoring of the volatile organic compounds (VOCs) produced by the fruit and investigation of biochemical reactions - with the overall goal of providing a suite of simple diagnostic checks to monitor fruit quality.

In this presentation, we will focus on the VOC bouquets from peach cultivars in an attempt to identify molecular markers for objective quality assessment.

Thermal desorption (TD) enables rapid and robust in-situ sampling of VOCs, on to sorbent tubes that can be subsequently capped for safe transport to the laboratory for analysis. Here, we use comprehensive two-dimensional gas chromatography coupled with time-of-flight mass spectrometry (GCxGC–TOF MS) to provide enhanced separation of these complex samples. The pre-concentration effect of TD, combined with improved separation and highly-sensitive detection by GCxGC-TOF MS provides a comprehensive chemical fingerprint in a single analytical run.

The VOC profiles at the time of harvest and after storage at low temperature will be compared and correlated with results from sensory evaluation.

Keywords: Food Science, Gas Chromatography/Mass Spectrometry, Thermal Desorption, Volatile Organic Compounds
Application Code: Food Science
Methodology Code: Gas Chromatography/Mass Spectrometry
It is interesting to know how the cell responds to a small external perturbation. However, it is highly complex to point out where is the change in the complex internal metabolic network of a cell. The ultrafast interconversions of highly unstable intermediates of those metabolic pathways make their quantification impossible. Interestingly, metabolic footprinting techniques particularly help to understand the active changes in the metabolic pathways by probing the exometabolites. The present investigation uses the kinetic data of exometabolites, obtained by HPLC-organic acid analyser column, to understand the predominant intracellular metabolic pathway of M. purpureus. The predominant set of metabolites obtained from the active metabolic flux pattern of M. purpureus helped to link substrates to the final product of fermentation. It has also helped us to visualize the insight of the culture conditions’ influence on the production of lovastatin in both static and dynamic culture conditions. It is further used to develop a pathway that connects the simple sugar to the final productlovastatin. Subsequently, the proposed metabolic cascade was subjected to metabolic pathway analysis to examine the consistency of the proposed metabolic cascade. Based on the dimension of internal metabolites and internal fluxes, elementary flux modes were established. These flux modes were modelled using dynamic macroscopic modelling. This dynamic model was solved using initial guess of products. The output profile of dynamic model for lovastatin and other metabolites were compared with the experimental kinetic data. It showed a good agreement between the proposed dynamic model and experimental data.
The use of grass and food waste as the source of bio-based chemical production and waste management is a long term goal for scientists. Grass, which contains nature’s most abundant renewable polymer, is considered among the most promising sources of biorenewable feedstock. The high growth rate of grass makes it an interesting resource to produce bio-based commodity chemicals in a sustainable manner. The aim of the present study is to develop fermentation conditions under which grass can be effectively converted into useful chemicals as directed.

In this study, fermentation of reed canary grass under different conditions was investigated. Different combinations of bacteria (Clostridium acetobutylicum (Cac)) and fungi (Neocallimastix californiae G1 (Neo G1) or Anaeromyces robustus S4 (Ana S4)) were tested for their ability to produce chemicals while growing on grass as a carbohydrate source. Two media, minimum medium (M2) and rich medium (MC) were also tested for their effects in fermentation process. The fermentation products were monitored by HPLC and MS which showed that (i) grass fermented with fungi produced mostly lactate, acetate, and formate; (ii) addition of Cac into the system, either simultaneously or by two-stage addition, yielded more alcohols, i.e. ethanol and butanol. The best condition for the production of lactate is by using fungi only in M2 media.

In this study we demonstrated how consortia can be designed to perform bioprocesses of interest circumventing the metabolic limitations of a single organism.

Keywords: Bioanalytical, Biological Samples, Biotechnology, HPLC
Application Code: Genomics, Proteomics and Other 'Omics
Methodology Code: Liquid Chromatography
Glycoproteins on the mammalian cell surface play important roles in the regulation of cell-cell communication, response to extracellular cues, and initiating intercellular signaling. Several diseases arise from aberrant surface protein glycosylation. Inhibitors of glycan synthesis and processing have been identified and extensively studied, including tunicamycin, which inhibits the transfer of N-acetylglucosamine-1-phosphate from UDP-N-acetylglucosamine to dolichol phosphate, and thus prevents the synthesis of the first lipid-linked oligosaccharides intermediate N-acetylglucosamine-pyrophosphate-dolichol, and castanospermine, which is an α-glucosidase I and II inhibitor that causes the glycan to retain three glucose residues, preventing the glycan from maturing. However, the effects of protein glycosylation and glycan maturation on cell surface glycoproteins remain to be explored. In this study, we systematically identify and quantify surface glycopeptides of MCF7 cells treated with tunicamycin or castanospermine by combining Stable Isotope Labeling with Amino Acids in Cell Culture (SILAC), copper-free click chemistry, and MS-based proteomics. We were able to identify 1090 glycopeptides from 509 glycoproteins located only on the cell surface, and for the experiment with castanospermine treatment, 614 glycopeptides and 306 glycoproteins on the cell surface were quantified with several glycoproteins being down-regulated. For tunicamycin, the effect is still being studied. For the first time, we systematically investigate the effects of glycan synthesis and maturation on glycoprotein secretion to the cell surface. This work will provide insights into cell surface glycoprotein trafficking, leading to a better understanding of surface glycoprotein functions and cellular activity.

Keywords: Mass Spectrometry, Peptides, Protein, Proteomics
Application Code: Genomics, Proteomics and Other ‘Omics
Methodology Code: Mass Spectrometry
Aptamers are gaining increasing interest for their applications as pharmaceuticals, biosensors, and affinity reagents. Unfortunately, these short oligonucleotides are often limited by current conventional selection processes that rely on combinatorial libraries whose amplification steps can significantly under represent certain secondary structures and create sequence bias. This is especially true for guanine rich sequences that are capable of forming G-quadruplexes (G4s). Recently, many proteins have been shown to interact with DNA secondary structures throughout the human genome, and new Chromatin Immunoprecipitation (ChIP-exo) techniques have made it possible to quantify these interactions at single-nucleotide-resolution on a genome wide scale. These sequences can then be screened \textit{in silico} for secondary structure formation and their protein affinities can be measured \textit{in vitro}. The protein Nucleolin (NCL) has been shown, by our lab and others, to interact with human genomic G4-forming-sequences \textit{in situ} and \textit{in vitro}. Furthermore, these sequences have been shown to have substantial regulatory significance on nearby genes. NCL plays a pivotal role in cancer as both a transcription factor and a cell surface receptor, and the protein has acquired notable attention as a therapeutic target for aptamers and antibodies. Here we use ChIP-exo to study NCL’s genomic interactions in the metastatic breast cancer cell line BT474. We have identified numerous potential G4 forming sequences that could be used as aptamers against NCL. Our approach also uncovers new genes that NCL interacts with, improving our understanding of the proteins role in cancer cell biology.
Violent crimes committed with modern automatic firearms have a number of residual items that are likely to be discarded at crime scenes. During preparation, the perpetrators of these crimes have had intimate contact with these components during magazine loading. This contact provides an opportunity to collect DNA samples from these discarded items that are directly linked to the perpetrators. Forensic DNA analysis by STR genotyping of individuals is a very powerful tool to assist law enforcement officers and prosecutors trying to properly identify and successfully convict criminal offenders. The combined DNA index system (CODIS) database allows for nationwide STR profile searches against samples from crime scenes with no known suspect. These benefits and successes have caused a near exponential increase in the use of forensic DNA testing. This increase in demand has overwhelmed crime labs, creating large backlogs of samples for DNA testing. Storage of these backlogged samples can create yet another logistical hurdle for resource strapped laboratories. In this study we chose to compare the efficacy of three collection devices for collecting touch DNA samples from decontaminated polymer and aluminum assault rifle magazines that were then loaded by test subject: traditional cotton swabs (Puritan), CEP cotton paper swabs (Fitzco), and nylon flocked swabs (Copan). We also determined the efficacy of a novel, room-temperature, storage device, the SwabSaver[registered] (FastForward Forensic, Madison, WI), to preserve biological samples for later testing over other room-temperature storage methods. To determine the efficacy of swab type and preservation method DNA quality was ascertained via both DNA quantification (PowerQuant[registered], Promega, Madison, WI) and STR genotyping performance (Powerplex[registered]Fusion, Promega).
Characterization of a potentially large number of metabolites in complex mixtures is a primary goal in metabolomics study. Application to biofluids and tissue samples offers insights into biochemical metabolic pathways and their role in health and disease. 1D $^1$H and 2D $^{13}$C-$^1$H HSQC NMR spectra are most commonly used for this characterization purpose. They yield quantitative information about each proton of the mixture, but do not tell which protons belong to the same molecule. Interpretation requires the use of NMR spectral databases, which naturally limits these investigations to known metabolites. Here, a new method is presented that uses complementary ion exchange resin beads to differentially attenuate 2D NMR cross-peaks that belong to different metabolites. Based on their characteristic attenuation patterns, cross-peaks could be clustered and assigned to individual molecules, including metabolites with multiple spin systems, as demonstrated for a metabolite model mixture and $[$i$]$E. coli$[$/i$]$ cell lysate.
Very Low Density Lipoprotein Receptor (VLDLR) is a member of the low-density lipoprotein receptor family expressed in various tissues and more importantly in vascular endothelium. VLDLR influences transendothelial migration of leukocytes through its interaction with fibrin. VLDLR has very high affinity for fibrin fragments. Interestingly, the N domain of fibrinogen upon interaction with VLDLR reduces inflammation related atherosclerosis. We are interested in understanding the VLDLR-fibrin N interaction and its role in inflammation related atherosclerosis. However, so far there is no structural information of VLDLR. Solution NMR studies have been performed to attain the structural details of VLDLR. VLDLR has a folded structure and almost all of the cysteines are found to form disulfide bonds based on the chemical shifts assignment. Disulfide bonds among all the 18 cysteines are important to preserve the functional integrity of VLDLR. Each of the VLDLR domains bind to Ca2+ and it is most stable in the Ca2+ bound form. Based on backbone relaxation dynamics study, it has been observed that the two N-terminal domains of VLDLR seem to diffuse together, while the third domain diffuses independently. Combination of complete structural information and dynamics of all the domains of VLDLR only and upon binding with fibrin N would provide better understanding of the biological significance of VLDLR-fibrin interaction and provide a step forward for designing better inhibitor of this interaction that can reduce inflammation.

Keywords: HPLC, NMR, Protein, Solution
Application Code: Biomedical
Methodology Code: Magnetic Resonance
Recent advancements in acquisition speed and sensitivity of modern mass spectrometers have made the quality of chromatographic separations paramount in all advanced liquid chromatography tandem mass spectrometry (LC/MS-MS) workflows. As the mass spectrometer samples faster and reaches deeper into the proteome, availability of new, high-purity precursors becomes the rate-limiting step for increasing the number of peptides sequenced. Here we explore how utilization of high pressure (>30K psi) for packing of in-house made capillary columns affects experimental peak capacity and therefore, results of shotgun proteomics experiments. We adopted and modified previously described set-ups for high pressure packing and manufactured columns at three pressures (3K, 20K, and 30K psi) in triplicates. All analyses were performed on an Orbitrap Fusion Lumos (Thermo Fisher Scientific), collecting MS/MS scans in the ion trap at ~36 Hz. Columns packed under higher pressure exhibited lower back pressure that permitted the use of longer than typical columns (40 and 30 cm, respectively). These columns were also remarkably uniform in performance and routinely afforded peak capacity of ~850 over 70-min separations. We observed ~10% increase in peptide identifications detected in 90-min LC/MS-MS analyses of whole yeast and human proteome tryptic digests as well as fractionated human cell line samples. Even greater improvements in the number of human peptides identified (~16%) were achieved over 180-min separations. The high pressure packed columns also demonstrated superior sensitivity, as evident by ~19% increase in peptide identifications in the analyses of small peptide loads (<100 ng) and enrichments of post-translationally modified peptides. Thus, our work strongly corroborates the advantage of high pressure column packing for LC/MS-MS and underscores significance of separations in the state-of-the-art proteomics.

This work was funded by P41GM108538 and R35GM118110 (awarded to J.J.C.).
The need for rapid and efficient high throughput metabolic phenotyping (metabotyping) in metabolomic/metabonomic studies often requires compromises to be made between speed and metabolome coverage. Here the effect of LC column length (150, 75 and 30 mm) and gradient duration (15, 7.5 and 3 min respectively) on the number of features detected when untargeted metabolic profiling of human urine using reversed-phase gradient ultra performance chromatography (UHPLC/MS), with and without ion mobility spectroscopy (IMS), has been examined. As would be expected, reducing column length from 150 mm to 30 mm, and gradient duration, from 15 to 3 min, resulted in a reduction in peak capacity from 311 to 63 and a similar reduction in the number of features detected from over ca. 16000 to ca. 6500. Under the same chromatographic conditions employing a UHPLC/IMS/MS, to provide an additional orthogonal separation, resulted in an increase in the number of MS features detected to nearly 20,000 and ca. 7500 for the 150 mm for the 30 cm columns respectively. Based on this limited study the potential of LC/IMS/MS as a tool for improving throughput and increasing metabolome coverage clearly merits further in depth study.
Quantitative metabolomic analysis with very high coverage is of utmost importance in metabolomics, but is very challenging due to great diversity in chemical and physical properties and huge concentration ranges of the metabolites. In this work, we report our study of combining four chemical isotope labeling (CIL) LC-MS methods to target different submetabolomes of human plasma samples with an objective of determining the overall metabolome coverage and quantification performance for blood metabolomics.

In this work, four chemical labeling reactions are applied to target different submetabolomes: dansylation for amines/phenols, base-activated dansylation for hydroxyls, DmPA bromide labeling for carboxylic acids and dansylhydrazine labeling for carbonyl metabolites. Each reaction can be conducted either with 12C-reagents or 13C-reagents. After LC-MS analysis, quantitative analysis can be achieved using the peak ratio of the individual peak pairs found in mass spectra and a labeled standard library is used for positive metabolite identification.

In human plasma analysis, in total, 8225 ± 59 peak pairs or metabolites were detected (2157±7 peak pairs in amines/phenols, 2429 ± 9 peak pairs in carboxyls, 2062 ± 21 peak pairs in carbonyls and 1577 ± 22 peak pairs in hydroxyls, n=3). Among them 217 metabolites could be positively identified. These results indicate that an unprecedentedly large number of metabolites could be detected using the integrated approach of combining four CIL LC-MS methods. The quantitative performance of these methods for plasma metabolomics is being evaluated. We will also present the results of putative identification of detected metabolite peaks based on accurate mass search against various metabolome databases.

Keywords: Bioanalytical, Derivatization, Liquid Chromatography/Mass Spectroscopy, Metabolomics
Application Code: Genomics, Proteomics and Other 'Omics
Methodology Code: Liquid Chromatography/Mass Spectrometry
Plants respond to environmental pollution with mechanisms that include immobilizing, chelating, and compartmentalizing toxins. Part of their defense mechanism is the production of small, metal-binding ligands called phytochelatins (PCs). These are small, thiol-rich peptides synthesized by plants in response to heavy-metal stress. Analytical techniques have been used to characterize the binding of heavy-metal ions in biological systems, but there is little known information about the production of PCs with a popular agriculture technique called aquaponics. Aquaponics is the practice of simultaneous plant and fish production, in a closed and recirculating system. Heavy-metal contaminants can be introduced into aquaponics via fish feed, and can become bioavailable to the plants in this agricultural system. To determine the presence of PCs in these systems, SEC-HPLC and RP-HPLC coupled to ICP-MS/MS was used. The distribution of heavy metals in root extracts at different molecular mass fractions was assessed by SEC-ICP-MS/MS. To evaluate the presence of PCs the proteinaceous fraction of the extracts was precipitated with TFA and Peptide-SEC-ICP-MS/MS was used with sulfur detection to determine total thiols and their coordination with metals in the extract.
Per- and poly-fluoroalkyl substances (PFASs) are synthetic organofluorine compounds which are extremely resistant to degradation due to heat, acids, or bases. They are present in a variety of products—including food packaging, textiles, and fire-fighting foams—and have been detected in the environment as well as in the blood of the general population. Additionally, PFASs are bioaccumulative and have been associated with various adverse health effects. This, combined with the persistent nature of PFASs, has prompted the USEPA to issue health advisory limits for two of the most common PFASs: PFOA and PFOS. In the recent year due to the US EPA health advisory issued in May 2016 at 70 ng/L in drinking water it has received much attention. Presented in this study are various workflows for the sample preparation and analysis of Perfluoroalkyl Substances in water as well as food samples using advancements in sample preparation techniques as well as chromatographic improvements coupled to LC-MS/MS.
Plasma based ambient desorption/ionization (ADI) sources for mass spectrometry (MS) generally use helium as the discharge gas due to the large reaction cross section of excited helium species with atmospheric gases, which ultimately leads to large reagent-ion densities. However, some studies have shown mixed-gas plasmas provide unique advantages. Previously, we showed that a mixed-gas flowing atmospheric-pressure afterglow (FAPA) source could provide enhanced ion signals, chemically cleaner mass spectra, or unique gas-phase chemistries. To further expand upon the unique advantages of a mixed-gas FAPA, fundamental understanding of the plasma processes is necessary.

In this study, optical characterization of mixed-gas FAPA sources are presented. The influence of molecular gas (O2, N2, or H2) addition on the optical emission of a helium-based FAPA was measured with an Avantes multichannel spectrometer capable of simultaneously measuring emission from 175 to 1100 nm. Spatially resolved emission was obtained by the use of a motorized translation stage. In general, addition of molecular gases noticeably decreased the helium emission intensities. Emission from OH* also decreased on addition of N2, H2, or O2 gases. Oxygen emission line decreased on addition of N2 and H2 whereas it increased first and decreased on addition of more O2. Furthermore, emission characteristics of N2*, N2+*, NO*, H (I), etc. with respect to molecular gas composition on He-FAPA will also be discussed. This information should be helpful to better explore the advantages and disadvantages of molecular-gas addition to FAPA source.

**Abstract Text**

Plasma based ambient desorption/ionization (ADI) sources for mass spectrometry (MS) generally use helium as the discharge gas due to the large reaction cross section of excited helium species with atmospheric gases, which ultimately leads to large reagent-ion densities. However, some studies have shown mixed-gas plasmas provide unique advantages. Previously, we showed that a mixed-gas flowing atmospheric-pressure afterglow (FAPA) source could provide enhanced ion signals, chemically cleaner mass spectra, or unique gas-phase chemistries. To further expand upon the unique advantages of a mixed-gas FAPA, fundamental understanding of the plasma processes is necessary.

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**Keywords:** Mass Spectrometry, Plasma Emission (ICP/MIP/DCP/etc.)

**Application Code:** General Interest

**Methodology Code:** Mass Spectrometry
Quantitative Analysis of Compounds Separated on Thin Layer Chromatography (TLC) Slides by Low-Temperature Plasma (LTP) Desorption/ionization Mass Spectrometry

Thin layer chromatography (TLC), a well-established technique for compounds separation, takes advantages of being very simple, fast, inexpensive, without any “memory” effects due to the renewing of TLC plate for each analysis. When coupling with mass spectrometry (MS), it allows fast identifying and characterizing the TLC spot, moreover, the ion intensity can be used for quantitative assessment.

The low temperature plasma (LTP) probe, a plasma-based ambient desorption/ionization source for mass spectrometry, introduced in 2008, has emerged as a frontier technology. This ionization technique possesses several advantages including relatively soft ionization, fast analysis, cost efficiency, little-to-no pretreatment requirement and capability of combining with any MS instruments with ambient inlet. Also, it has the advantage of more efficient ionization of low molecular weight compounds over a relatively wide polarity range compared to spray-based techniques.

Here, the development of direct coupling between TLC and LTP desorption/ionization mass spectrometry for fast characterization and quantitation of a large variety of analytes will be presented. The recent results demonstrated the successful employment of LTP for TLC plate analysis. The effect of several TLC-LTP-MS experimental conditions on the analytical performance of a variety of compounds will be shown. This TLC-LTP-MS technique is attractive because it allows inexpensive, fast and quantitative analysis, and can be applied in synthetic organic laboratories and forensic sciences.

Keywords: Characterization, Mass Spectrometry, Quantitative, Thin Layer Chromatography
Application Code: Process Analytical Chemistry
Methodology Code: Mass Spectrometry
Automobile engine oil is composed of base lubricating oil plus an additive package of carefully selected chemical components to protect this base oil. The combustion events of an internal combustion engine combined with heat, friction, and contamination from water and fuel eventually wear out the additive package leaving the base oil susceptible to degradation. Oxidation is the primary method for this oil degradation and is routinely measured by infrared spectrometry or titration techniques. DART – TOFMS, direct analysis in real time-time of flight mass spectrometry, offers a rapid precise monitoring of individual chemical components of the additive package and the oil.

Analysis of oil samples taken at 20-hour intervals of a 100-hour engine dynamometer test with direct analysis in real time (DART) time-of-flight mass spectrometry has been used to identify and monitor several key components of the engine oil. By varying the ionization modes of the DART, changes to the antioxidants, anti-wear additive components and the base oil were successfully monitored.
Chromium is found predominately as trivalent chromium (Cr(III)) and hexavalent chromium (Cr(VI)). In low doses, Cr(III) is an essential nutrient required for proper metabolism and is often included in dietary supplement formulations. However, Cr(VI) is highly toxic and is absorbed more readily than Cr(III) by the lungs, gut, and skin. The CDC identifies Cr(VI) as a toxin, mutagen, and carcinogen proven to cause developmental and reproductive toxicity. California’s Proposition-65 sets the maximum allowable dose level of Cr(VI) at 8.2 µg/day. However, only total chromium concentrations are routinely determined and used for dietary supplement labeling. Assessment chromium’s benefits and associated risks requires an accurate analytical method that is capable of both Cr(III) and Cr(VI) quantification. Molecular speciated isotope dilution mass spectrometry (SIDMS) with direct mathematical deconvolution is a unique technique that utilizes two different isotopically-enriched spikes during sample preparation to correctly identify and determine concentrations of both the Cr(III) and Cr(VI) species. The concentrations and stability of Cr(VI) in a variety of dietary supplement samples were determined by using a modified microwave-enhanced alkaline extraction protocol integrated with SIDMS, IC-ICPMS analysis, and data processing according to EPA Method 6800, update V, 2015. Recent results will be presented with discussion of Eh and pH phase diagram stability of Cr(VI) in dietary supplement samples. Over half of the prenatal dietary supplements tested had quantifiable Cr(VI). Therefore, this test method should be required by the US FDA, US Pharmacopeia, and other world health organizations as a routine public safety protocol.
Development of a rapid and reliable analytical method for the uptake and toxicity of nanoparticles (NPs) in cells is becoming increasingly important, especially in assessing the NP uptake distribution throughout a population of cells. A novel single cell (SC)-ICP-MS sample introduction system with a high transport efficiency allows for the quantification of the dissolved metal, intrinsic metal, and NP concentrations within intact cells, simultaneously providing a distribution of the mass uptake of these analytes on a per-cell basis within a few minutes. While the full capabilities of SC-ICP-MS are still being explored, this study focused on evaluating the method’s strengths and limitations for reliably analyzing the uptake of citrate coated silver NPs by the yeast strain \textit{Saccharomyces cerevisiae}. Cells were dosed with silver NPs (AgNPs) for a period of time and then prepared for SC-ICP-MS analysis. Experimental data showed that the transport efficiency can reach 30-90\% for nanoparticles and about 30\% for yeast cells. Detection limits obtained were 16nm silver NPs, 0.03 ug/L dissolved silver, and about 2,000 cells/mL. The distribution of AgNPs across the cell population, as well as the cell concentration in the sample, were obtained during analysis. This allowed for AgNP uptake over time in yeast cells to be observed. This technology is expected to have potential broad applications for rapid monitoring of NPs and cytotoxicity at the single-cell level, as well as for monitoring NP applications in drug delivery. The detailed experimental procedures and data will be presented at the conference.
Coupling solid-phase microextraction (SPME) in various configurations and geometries to ambient mass spectrometry (AMS) have enabled rapid screening and quantitation of multiple compounds of biological, clinical or forensic relevance in complex biological matrices. In this study, we present an in-house built desorption electrospray ionization (DESI) source equipped with a custom-made holder that allows accommodating SPME fibers of different lengths with the objective of providing spatial resolution information from three-dimensional objects. Unlike previous space-resolved studies with SPME, where there is need of slicing the wire to small portions and subsequent independent runs by liquid chromatography, the combination of SPME fibers and DESI not only accelerates the speed of analysis but also allows truly investigating the uneven distribution of endogenous and exogenous compounds in tissue as this AMS technology facilitates accurate small micro-desorptions across the coated wire and without additional steps. Using uniformly thin coated fibers allowed obtaining both qualitative and quantitative information about laminar distribution of analytes within the studied system. As a proof-of-concept, the system was evaluated towards the detection and quantitation of several pharmaceuticals spiked on a multilayer agarose arrangement. The suitability of the presented technique to characterize more complex systems such as brain tissue was further evaluated by spatially-resolved analysis of endogenous compounds in rat brain. The coupling of this technologies to hybrid high resolution mass spectrometer (HRMS) enabled unambiguous identification of detected compounds by providing both accurate mass and fragmentation spectra, what contributed to even better characterization of the studied system in one run. The presented approach can be seen as a complementary tool to existing AMS technologies for tissue analysis when 3D in vivo information is desired in the surgery room.

**Keywords:** Mass Spectrometry, Neurochemistry, SPME, Time of Flight MS

**Application Code:** Neurochemistry

**Methodology Code:** Mass Spectrometry
Release of engineered nanoparticles (NPs) into environment is increasing with the rapid development of nanotechnology and the corresponding growing applications. Investigation of the fate, transportation, environmental toxicity of released NPs are in urgent need. Cerium dioxide nanoparticles (CeO2 NPs) are one type of widely used NPs due to their unique oxidative/reductive and catalytic properties. Once the CeO2 NPs are released into the environment, soils are likely to be their primary repository. Detection and characterization of CeO2 NPs in soil is a challenge due to their low predicted environmental concentrations and the high natural background of Ce-containing minerals, thus the fate of the CeO2 NPs after released into soils is largely unknown. In this study, we demonstrate the development and validation of a method for the extraction and determination of particle size and concentration of CeO2 in soils. The method is composed of extracting CeO2 NPs from soils using tetrasodium pyrophosphate (TSPP) aqueous solution and characterization of aqueous extracts through single particle ICP-MS. We assessed the efficacy of the extraction by varying the concentration of TSPP solutions. Our results showed that the optimal method employed 5 mM TSPP (1:100 soil-to-reagent ratio), with ultra-sonication to enhance particle dispersion, could efficiently extract CeO2 particles out from both non-aged and aged CeO2 NP (30-50 nm)-dosed soil samples with ~100% recovery yield. In addition, the size of the dosed CeO2 NPs in aged soil samples got slightly increased, in comparison with that in non-aged soil. Overall, the developed method was capable of extracting and accurately measuring the size and concentration of CeO2 NP in soils.

Keywords: ICP-MS, Particle Size and Distribution, Soil
Application Code: Environmental
Methodology Code: Mass Spectrometry
The growing demand for portable mass spectrometers has increased pressure on miniaturization of all the components. One of the biggest challenges is implementation of compact electronics with stable resonant circuit to enable highly linear RF scan. This is particularly difficult at higher RF voltages required to achieve high mass ranges. Since many in-field applications (e.g. food safety, security, etc.) require identification of specific compounds, scanning of the full mass range is not essential. Therefore, identification of 3 or 4 characteristic mass fragments may be sufficient allowing much faster identification of target substances. This can be achieved by running a mass analyzer in a non-scanning mode by using fixed RF and DC voltages for isolation of individual mass fragments.

This work presents simulation results for a non-scanning linear ion trap (LIT) using the LIT2 program based on the boundary element method (BEM). The focus is on improving sensitivity and resolution as these are difficult to achieve at high levels in a non-scanning mode. This was done by varying the parameters of a DC isolation pulse. Five main parameters of the pulse are varied: pre-delay time (between ion injection and initial trapping), pulse ramp-up time, pulse flat time, pulse ramp-down time and post-delay time (final ion trapping before ejection). It has been found that delay and ramp times greatly affect sensitivity, while flat time had major effect on resolution.

After optimization, unit resolution at 50% of the peak height was achieved up to 500 Da mass range with UV ratio at 99.7%. Total scan time per mass fragment was 1 ms, which allows analysis of hundreds of compounds in a sample within a second. This can be useful for applications where thousands of samples are scanned per day for large number of different compounds.

Keywords: Food Safety, Ion Trap, Mass Spectrometry, Portable Instruments
Application Code: Food Safety
Methodology Code: Mass Spectrometry
Development of a Portable TOFMS for On-Site Fault Diagnosis of SF6 Insulated Switchgear by Detection of Decomposition Products

Sulfur hexafluoride (SF6) gas insulated switchgear (GIS) is an essential electric equipment in a substation, and the concentration of the SF6 decomposition products are directly relevant to the security and reliability of the substation. The detection of SF6 decomposition products can be used to diagnosis the condition of the GIS. The decomposition products of SO2, SO2F2, and SOF2 were selected as indicators for the diagnosis. In our work, a portable time-of-flight mass spectrometer (TOFMS) was designed to perform online GIS failure analysis. A RF VUV lamp was used as the photoelectron ion source. With a miniature orthogonal acceleration, reflectron TOF mass analyzer and a set of well-designed ion transmission optics, the portable TOFMS achieves a mass resolution of more than 1500 m/Δm with only a 25-cm long drift region, and the limits of detection (LOD) of SO2 and SO2F2 were both achieved 1 ppm in 100 sec, and the sensitivity is estimated to be at least 10-fold more sensitive than the previously design. The high linearity of SO2, SO2F2 at the range of 5–100 ppm has excellent linear correlation coefficients at 0.9951 and 0.9889, respectively. The whole system weighs only 20 kg with the size of 37×33×23 cm including battery, consumes only 70 W. Experiments were carried out on site at a gas-insulated substation under real-world conditions. The analysis time for each sample is only 100 s while the sample consumption is within 100 ml. The results revealed the strong capability of this portable TOFMS to withstand the environmental conditions, and a wide application prospect on establishing an early-warning for the failure of the GIS.

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Keywords: Detection, Instrumentation, Mass Spectrometry, Sulfur
Application Code: Safety
Methodology Code: Mass Spectrometry
Mass spectrometry (MS) is a key technology for the analysis of petroleum and its products. Time-of-flight MS (TOFMS) combines the merits of a high acquisition speed and a high mass resolution. Therefore, TOFMS is very versatile for the hyphenations with different interface technologies which are crucial to deal with the different physical properties like boiling point, cracking behavior and polarity of petroleum. Heavy petroleum fractions from petroleum like bitumen are most challenging and even if the net product for this matrices is not very high they arise in tons and did become an important material e.g. for street construction. The characterization and specification of such products for industrial usage is mostly based on general physical properties. However, these parameters only reflect the actual workability of the matrices but not its long term chemical behavior like aging or vulnerability.

For this study we will demonstrate the applicability and versatility of high resolution (accurate mass) multi-reflection TOFMS for the analysis of such matrixes. Different Bitumen as well as high boiling point petrochemica cuts are artificial aged before and after SARA fractioning. For a comprehensive investigation of the chemical composition and aging dynamics, different ionization methods (70eV EI and VUV-SPI) as well as different sample introdution methods (Direct insertion probe (DIP), simultaneous thermal analysis (STA), comprehensive two-dimensional gas chromatography (GC×GC)) are applied. GC×GC allowed the most comprehensive analysis with access to individual isomers/isobars of the matrix and a direct link to common mass defect information of the accurate mass TOF. DIP could almost scope the complete boiling range of the products while STA (evolved gas analysis) allowed also the investigation of possible cracking products. The TOFMS was operation with R>25.000 if pre-separation could be applied (GC×GC or STA×GC) and R=50.000 in case of DIP.

Keywords: Gas Chromatography, Petroleum, Thermal Analysis, Time of Flight MS
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Mass Spectrometry
Laser Ionisation Mass Spectrometry Experiments in Manchester (UK): Planetary Science, Nuclear Physics and Food Applications

A laser ionisation mass spectrometry is thriving in Manchester. We have developed several laser facilities that are tackling the problems ranging from analysis of primordial noble gases extracted from meteorites, chondrules and extraterrestrial samples returned by recent NASA missions (e.g. Genesis and Stardust), detection of rare short-lived isotopes produced in minute quantities at the isotope separator of ISOLDE, CERN and to authenticity analysis of oils and milk samples collected in remote regions of developing countries as a part of UN missions.

The developed technology incorporates laser desorption/ heating and cryogenic sample preconcentration, a range of tunable pulsed (ns or fs) lasers coupled into the ion source of time of flight, magnetic sector or accelerator beamline facilities. The method is suitable for applications requiring a high sensitivity. E.g. a detection limit of <100 atoms/ sample have been demonstrated during analysis of radiogenic 81Kr from meteorites with precision and reproducibility of ~1 % for the major isotopes.


Keywords: Instrumentation, Isotope Ratio MS, Mass Spectrometry, Nuclear Analytical Applications

Application Code: Other
Methodology Code: Mass Spectrometry
Platinum-based drugs are among the most active antitumor reagents in clinical practice and nanoformulations of active components can prevent uncontrollable drug activation and minimize systemic toxicity. In this study, platinum-gold nanostars (Pt-AuNS) is developed as a novel prodrug nanoplatform for remote control of antitumor activity toward different cancer cells. Upon near-infrared light illumination, Pt-Au metal species were released from the prodrug Pt-AuNS and exhibited high anticancer efficacy through a form of programmed cell death known as ferroptosis. This process is characterized by the accumulation of lipid peroxidation and lethal reactive oxygen species (ROS) and can be pharmacologically inhibited by iron chelators (e.g., deferoxamine) and lipid peroxidation inhibitors (e.g., ferrostatin and liproxstatin). The observation of a significant depletion of GSH/GSSG was also consistent with the fact that the released metal species could induce the formation of ROS, causing an oxidative cell death. Together, our findings demonstrate that ferroptosis can be targeted by photoactivation of platinum-gold nanostars and may have therapeutic potential in future clinical applications.
The current treatment options available for those suffering from Inflammatory Bowel Disease (IBD) have shown to be insufficient in significantly improving the quality of life of patients, as they have only focused on fighting the inflammatory response while ignoring the intestinal damage present. Mesenchymal stem cells (MSCs) exhibit the ability to both repair tissue, as well as act as anti-inflammatory agents. While the number of MSCs necessary to achieve these desired effects has led to venous complications from such large dosages, we believe more efficient delivery of the MSCs could help to overcome these complications. We hypothesize that targeting the MSCs to the intestinal vasculature, through the use of a peptide-dendrimer vehicle, will increase delivery efficiency to the injured sites and lead to both tissue repair and lessened inflammation. This increased efficiency would mean a smaller needed dose of MSCs, overcoming both the aforementioned venous complications as well as minimizing potential side effects. Based on our preliminary data, the MSCs in complex with the dendrimer and targeting protein exhibit increased homing to the intestine in a mouse model of colitis. We wish to continue to develop this targeted nanocarrier method of delivery to treat intestinal inflammation utilizing a mouse model of colitis. In addition, we wish to study the effects that the MSCs have during periods of inflammation on epithelial barrier function, epithelial stem cell dynamics, and epithelial cell proliferation. The use of colonoids will help us to assess these aspects of the experiment. This project may lead to improved therapies that can help to improve the quality of life for patients with IBD.
Since its discovery, the use of antimicrobial drugs has been globally widespread, both in human and in veterinary medicine, commonly used as therapeutic agents to combat infectious diseases of the bacterial type. In the veterinary case, these are also used as prophylactics for the prevention of infections derivate from stress states. Enrofloxacin (ENRO) and its active metabolite Ciprofloxacin (CIPRO) are antibiotics belonging to the quinolone family. In this context, the present work proposes the formation and characterization of polymeric nanoparticles charged with ENRO and CIPRO by the formation of interpolymer complexes, using complementary charge polymers containing important biological properties, such as mucoadhesivity, biocompatibility, and biodegradability as chitosan, chondroitin sulfate and hyaluronic acid. The stabilization and formation of such complexes is mainly due to electrostatic interactions between the polysaccharides and the drug.

Z-sizer results revealed the formation of nanoparticles with size ranging from 250 to 350 nm, with a zeta potential between 35 and 40 mV and a low polydispersity index of 0.15. The release of the drug from the nanoparticles was evaluated by means of the diafiltration technique, a technique that allows the separation of low molecular weight species from high molecular weight species, being able to determine the binding and efficiency of the drug to the nanoparticles. The quantification of the drug is performed by HPLC-ESI-MS. Finally, the polymer nanoparticles loaded with these drugs could be considered as powerful tools for the treatment of bacterial infections, improving the delivery of these drugs and their absorption properties.

The authors are grateful for the funding received to Fondecyt No 11150919 and 1150899.

References:

Keywords: Liquid Chromatography/Mass Spectroscopy, Nanotechnology, Pharmaceutical, Polymers & Plastics
Application Code: Nanotechnology
Methodology Code: Liquid Chromatography/Mass Spectrometry
Abstract Text

Sub-visible particle characterization has become an important method in assessing drug substance and drug product stability. However, the current USP protocol does not require any characterization of particles below 10 μm. This greatly hampers our ability to properly assess protein stability as a function of particulate formation and obscures most sub-visible particles and various morphological properties. In this study, we assess the capabilities of the new FlowCam Nano from Fluid Imaging Technologies, in conjunction with a pumping study that focuses on particulate formation generated during fill operations. Initial experiments show that the FlowCam Nano can generate highly resolved images of nanoparticulates, allowing the simultaneous assessment of particulate morphology for nano and microparticulates. This is extremely useful, as currently unpublished data (for the pumping study) show that as a function of post-pumping agitation, a significant increase in microparticle concentration coincides with a significant depletion of the nanoparticle concentration. This suggests that agglomeration (and probably not nucleation) is the more likely mechanism for larger microparticulate formation. The ability to characterize the nano and microparticulate morphology simultaneously will allow us to better assess the cause of particulate formation (by observing the transition of nanoparticles to microparticles and seeing if specific morphological properties of larger nanoparticulates are conserved in microparticulates). The FlowCam Nano is sensitive enough to assess differences in the colloidal stability of a drug product in different formulations. These capabilities are very useful in assessing drugs in the substance (e.g. optimizing drug formulations) and product (e.g. assessing particulates post fill & finish operations) phases.

Keywords: Biopharmaceutical, Imaging, Nanotechnology, Particle Size and Distribution

Application Code: Nanotechnology

Methodology Code: Process Analytical Techniques
Pollution of water, air and soil, which are directly or indirectly communicating with living things in parallel with the industrial technology that develops in order to provide a living environment in better conditions to a growing population, is an important toxicological problem. In the event of uncontrolled growth of production, uncontrollable urbanization, uncontrolled industrialization, acid rain, the formation of solid, liquid and gaseous waste, the use of random chemical substances and similar human activities rise above the natural renewal capacity, our wellness areas are adversely affected. The most important pollutant source is industrial and domestic wastewater. Since wastewater should have limits for physical (suspended solids, color), biological (bacteria and species) and chemical pollutants (organics, heavy metals), for this reason it must be thoroughly refined before being discharged to water basins with an indispensable prescription for the next generation, so all should be analyzed for all kinds of toxic substances by the control laboratories of the institutions. However, these analyses have taken place in the literature with features such as ease of use, fast response, repeated use, reliable high precision results in the use of sensors for this purpose in order to facilitate the process. For this purpose, this work has been based on the preparation of nanostructure-based adsorbents and their use in metal determination. Therefore; silver nanoparticle-coated activated carbon was synthesized via green chemistry and applied for solid state modification. Bare and modified surfaces were characterized by microscopic and electrochemical techniques. Metal adsorption capacities of coated activated carbon on solid electrode surfaces have been evaluated by electrochemical determination of transition metals. The effects of the presence of the nanoshell have been discussed by metal adsorption of activated carbon.

**Keywords:** Electrochemistry, Electrode Surfaces, Nanotechnology, Sensors

**Application Code:** Nanotechnology

**Methodology Code:** Electrochemistry
Nanotechnology, the area of science, focused on the control of matter in the nanometer scale, allows ground-breaking changes of the fundamental properties of matter. In recent years it has a profound impact on bioproduction systems, synthetic biology, medical diagnostics and therapy, drug delivery. This special issue is dedicated to the overview of the control of biosystems at the molecular- and nanoscale. Nowadays the nano level is the most advanced, both in scientific knowledge and in commercial applications. From the point of this view this study is one of the nanobiotechnological research based on the biological application of modified surfaces which is designed as active areas with nanoscale structures providing unique properties. The aims of this study were: (i) to synthesize silver nanoparticles via green chemistry and their spectroscopic and microscopic characterization, (ii) to use of nanoparticles for the modification of surfaces by immobilization process, (iii) to modify surfaces (after the 2nd treatment) by electrochemical deposition of copper ions, (iv) to characterize bare and modified surfaces and (v) to use the modified surfaces for electrochemical determination of uric acid.

Nanoparticles were characterized by UV-Vis spectroscopy and also scanning electron microscope. The electrode surfaces were modified with nanoparticles by physical adsorption. The modified surfaces were covered with copper by electrochemical deposition applied with cyclic voltammetry. All surfaces were characterized by electrochemical techniques and scanning electron microscope. A fast and sensitive uric acid electrochemical sensor was fabricated by being electrodeposited copper onto silver nanoparticles-modified glassy carbon electrode. The sensor had excellent stability, fast response, ease of construction and utilization for uric acid determination.

**Keywords:** Bioanalytical, Electrochemistry, Nanotechnology, Sensors

**Application Code:** Nanotechnology

**Methodology Code:** Electrochemistry
Many human diseases are caused by viral infection. Enterovirus 71 is a single RNA virus, which mostly infect children and teenagers. Chemical synthesized antiviral molecular drugs are costly and ineffective due to their poor biocompatibility, susceptibility to drug resistance and complex synthesis procedure. Carbon quantum dots (CQDs) have proven to have high biocompatibility, excellent water solubility, fluorescence, low cytotoxicity and low cost, which can greatly increase their applicability. Curcumin has been proved to have multiple functions including antioxidation, antibacterial, anti-cancer, anti-virus, many studies also demonstrated its use in drug treatment. In this study, we use dry heat curcumin to one-step synthesize curcumin carbon quantum dots (CQDcurs) with diameter of 2–4 nm. Fourier-transform infrared spectroscopy is used to characterize if curcumin functional groups remain after dry heating. CQDcurs were shown to have low toxicity in rhabdomyosarcoma cell (RD cell). Through the use of cytopathic effect and plaque assay, CQDcurs were shown to have the best effect in post-treatment. Additionally, TCID50 of CQDcurs is calculated to be 1 µg/mL, which is 25 times lower than curcumin. In this study, we synthesized CQDcurs a novel and simple CQDs. CQDcurs has not only retain some structural integrity of curcumin, but also preserved the antiviral properties of curcumin. Furthermore, unlike curcumin it is highly soluble. We believe CQDcurs has great potential as a novel anti-viral nano drug.

Keywords: Nanotechnology
Application Code: Nanotechnology
Methodology Code: Chemical Methods
Chemiluminescence (CL) is a commonly used detection technique in biology and is usually catalyzed by horseradish peroxidase (HRP) conjugated antibodies. In chemiluminescence, energy is released by a chemical reaction, which triggers light generation. In recent years, chemiluminescence has been widely used in environmental monitoring, clinical diagnosis and food safety due to its highly sensitive, fast and simple nature. However, chemiluminescence suffers from weak signals, short luminescence time, low intensity and a lack of reproducibility due to its rapid reaction time. In order to solve these problems, enhancers were developed and added to the original chemiluminescence system, also known as, Enhanced Chemiluminescence (ECL) system. Compared with the conventional chemiluminescence, enhancers can easily reacts with HRP-I (Oxidized HRP intermediate I) to increase the yield of free radicals; therefore, is more effective at increasing the luminous efficiency. However, the enhancers had some disadvantages in practical applications. Therefore, the purpose of this project is develop a new enhancer based on carbon nanomaterials and apply them to chemiluminescence system. The carbon nanomaterials (CNMs) are synthesized by calcination of phenolic molecules and halide ions. This method can be used to synthesize carbon nanomaterials with different functional groups on the surface. The HRP-catalyzed chemiluminescent oxidation of luminol can be used in many biological analysis method, such as western blots. We expect to develop an enhancer which one not only have the usual function of the enhancer, but also participate in the catalytic reaction of the chemiluminescence reaction. CQDs’ large surface area can increase the electron transfer rate and thereby increase the chemiluminescence reaction, achieving higher sensitivity for the detection of pathogens (pathogen) or biomarkers in cancer cells.

Keywords: Luminescence, Nanotechnology
Application Code: Nanotechnology
Methodology Code: Fluorescence/Luminescence
The most usual procedure utilized to detect Zika virus is based in the detection of its RNA by polymer chain reaction. The utilization of gold nanoparticles appears as an alternative. Methods to isolate antibody or antigen have already been developed, and both biomolecules can be used to modify metallic surfaces and used as probes. Considering gold nanoparticles and gold nanometric films as alternatives to detect Zika virus, we modified gold nanoparticles with Zika virus antibody and we utilized two different strategies to observe Zika antibody-NS1 protein interaction. In the first case, to two different gold nanoparticles suspensions modified with Zika virus antibody ([AB]= 0.01µg/mL) were added bovine serum albumin (BSA) ([BSA]= 0.01 µg/mL) and Zika virus NS1 protein ([NS1] 0.01µg/mL) (Figure 1A). This approach allowed the detection of the antibody-NS1 interaction by plasmonic coupling among nanoparticles, while BSA (a common protein in blood and potential interferent) does not present same behavior. In the second case, NS1 protein was utilized to modify SPR gold film, and the gold nanoparticles with antibody were interacted with this film as well antibody without any modification. The interaction NS1 protein-antibody is observed by angle shifting seen by SPRS (Figure 1B). SPRS measures indicate that binding of AB to AuNps surface decreases around 15 % of AB-NS1 interaction. These factors indicate that gold nanoparticles can be used to detect Zika virus NS1 protein and suggest a possibility to use these methods as alternatives to detect Zika virus.

Figure 1. (A) UV-Vis spectra of gold nanoparticles (A) modified with Zika antibody (black), modified with Zika antibody in the presence of BSA (red) and modified with Zika antibody after adding NS1 protein and (B) SPR spectroscopy curves obtained by the interaction of NS1 protein in the surface of gold nanometric film with antibody (black) and gold nanoparticles modified with antibody (red).
Gold nanoparticles can provide a simple, easy-to-use, inexpensive, point-of-care (POC) disposable with a fast diagnostic for detect dengue virus. However, gold nanoparticles have a predisposition to form aggregation. In this article we studied two previous methods commonly described in the literature to prevent the formation of aggregate by studying pH and addition of surfactant as surfactant. The characterization of the nanoparticles was performed using UV-Vis, TEM and analysis through the ImageJ program. We found that AuNP mixed with surfactant offer the most stable condition in aqueous solution compared to pH dependence. Ours result have maintained excellent dispersivity along of the year.
Regulation of specific genes is a novel approach to treat many diseases including hereditary ones. Morpholinos antisense oligonucleotides, synthetic nucleic acids, are neutral molecules with high target specificity and resistant to nuclease degradation. Most commonly delivery vehicles cationic liposomes and polymers interact with the negatively charged nucleic acids electrostatically. However, morpholino antisense oligonucleotides are neutral and these mentioned cationic materials show poor efficiency delivering the morpholinos. In this study, morpholinos were embedded into a tile shaped DNA origami structure for gene silencing. The DNA tiles have sticky ends and can be modified through complementary oligonucleotides. The nanostructures were modified with AuNPs to increase the cellular uptake. The morpholino embedded DNA tile-AuNPs nanostructures were used to deliver morpholino oligos to breast cancer cells for silencing of HER2, ER, Ki-67, and EGFR genes in breast cancer cells. Effective silencing of the genes was obtained and inhibition of breast cancer cell proliferation was observed after silencing of the targeted genes. The authors acknowledge the financial support from TUBITAK (Project 115Z426).

**Keywords:** Biotechnology, Gene Therapy, Nanotechnology, Nucleic Acids

**Application Code:** Nanotechnology

**Methodology Code:** New Method
Photodynamic therapy (PDT) is a non-invasive, high spatiotemporal specificity, and high efficiency therapeutic method that has been extensively studied. However most of the photosensitizer (PS) used in PDT can only be excited by UV/Vis light, which has a short tissue penetration and can damage cells. In this regard, upconversion nanoparticles (UCNPs) have been utilized as energy transducer to transfer near-IR light to lower wavelength that can be absorbed by PS. Here we designed an aptamer-based multifunctional ligand that can covalently load PS to the surface of UCNP, and make the UCNP a biocompatible and cancer cell-targeted nanodrug. The as-built PDT nanodrug has been shown to be selectively internalized into cancer cells and has a highly efficient and selective cytotoxicity in cell study.
A highly efficient nanozyme system, termed hollow multipod Cu(OH)2 superstructure (HMPS), has been developed via direct conversion from irregular nanoparticles. The HMPS displayed body size around 150 nm and branch lengths in the range of 150~250 nm. Based on the excellent catalytic property of HMPS, we developed a simple and highly sensitive colorimetric assay to detect urine glucose, and the results are in good agreement with hospital examination reports.
The search for efficient light harvesting materials has resulted in significant research interest in plasmonic metal nanostructures, due to their ability to support localized surface plasmons. Non-radiative decay of localized surface plasmons results in the production of hot charge carriers and the generation of heat, both of which can affect the efficiency of plasmon-mediated photoelectrochemical processes. To overcome the challenge of decoupling the impacts of each effect on the measured photocurrents, we develop a methodology for independently exploring the roles of hot carrier and heat generation in plasmon-mediated photoelectrochemical processes using scanning electrochemical microscopy (SECM). Light is used to drive a redox reaction at a plasmonic substrate, while an ultramicroelectrode tip is positioned close to the substrate to read out both the reaction products and the mass transfer rate of the redox species, which depends on the hot carrier generation and local heating, respectively. By controlling the potential at the tip and substrate electrodes, the roles of plasmon-driven hot carriers and local heating can be isolated and investigated independently. The effects of the irradiance and the excitation wavelength on the hot carrier and heat generation are explored respectively. The SECM approach is suitable for probing a variety of photoactive structures used in photovoltaic and photocatalytic devices.

**Keywords:** Electrochemistry, Fuels\Energy\Petrochemical, Laser, Temperature

**Application Code:** Nanotechnology

**Methodology Code:** Electrochemistry
Bipolar electrochemistry is a powerful tool capable of localized and asymmetric electrodeposition on nano-objects in the absence of an electrical contact. It offers a precise way to grow oxide particles on 3D substrates, but often requires high voltages, upwards 100 V. We will be presenting a synthesis method that employs bipolar electrochemistry to form manganese dioxide nanoparticles. In our work, two electrodes are placed on either side of a gold-nanotube membrane and a voltage is applied (1-4 V). Redox reactions, one cathodic and one anodic, occur at either end of the nanotube. The anodic reaction is chosen such that it forms MnO\(_2\) which is selectively electrodeposited at one pore orifice (Figure 1). Manganese oxides are interesting materials for sensing, catalysis, supercapacitor and battery applications. Their well-defined porous structures exhibit high specific surface areas and lattices that allow the intercalation of ions, such as lithium. We will present the mechanism of formation and growth rate of these MnO\(_2\) structures on gold nanotubes, as studied by electrochemistry and complementary techniques. We will also report chemical and electrochemical characterizations of these nanoparticles in order to address the performance of this material for applications in supercapacitors and batteries.

The work was supported as part of the Nanostructures for Electrical Energy Storage (NEES), an Energy Frontier Research Center funded by the U.S. Department of Energy, Office of Science.

**Keywords:** Electrochemistry, Fuels\Energy\Petrochemical, Materials Characterization, Nanotechnology

**Application Code:** Nanotechnology

**Methodology Code:** Electrochemistry
In recent years, there has been significant research on the use of nanoparticles (NPs) for a wide variety of applications in the life sciences, energy and environmental fields. It is known that the characteristics of the surface coating determine many NP properties, notably stability, solubility and catalytic activity. This presentation describes the use of collision electrochemistry for studying the effect of capping agent on the redox behavior and catalytic activity of polyethylenimine-capped silver nanoparticles (PEI-AgNPs). We show that PEI-stabilized AgNPs exhibit significant changes in surface charge, oxidation and catalytic activity as a function of pH. Chronoamperometric signals respond rapidly to pH-induced changes of the PEI chains at the AgNP’s surface. Collision electrochemistry allows for rapid screening and catalytic activity evaluation of NPs, demonstrated in this work with methylene blue and PEI-AgNPs. These observations show that NP collision electrochemistry can be used in addition to spectroscopy and microscopy for studying conformational changes of capping agents and for evaluating the relationship between the surface properties and catalytic activity. This study provides fundamental information about polyethyleneimine’s surface characteristics, and activity in various environments. The method can be used as a general strategy for correlating surface properties of capping agents and screen NPs for their electrochemical, redox and catalytic properties.

Keywords: Materials Characterization, Nanotechnology, Single Molecule, UV-VIS Absorbance/Luminescence
Photocatalytic reduction of CO$_2$ offers a great potential for renewable energy storage. There is a tremendous interest in developing photocatalysts for CO$_2$ reduction in order to lower the energy needed to convert CO$_2$ to energy dense fuels. Cuprous oxide (Cu$_2$O) has good optical and magnetic properties and is a p-type semiconductor with a direct bandgap of 2.17 eV. Cu$_2$O has gained attention for the conversion of solar energy into electrical or chemical energy. Currently, Cu$_2$O is deposited onto substrates by several methods, such as thermal oxidation, chemical vapor deposition, electrodeposition, sputtering, and anodic oxidation. In this research, we describe an electrodeposition technique for synthesizing Cu$_2$O attached to Ag dendrites for use as photocatalyst for CO$_2$ reduction. Our work shows that Cu(I) is preferentially deposited onto Ag dendrites by electrochemical methods. The electrochemical modification of Ag dendrites with Cu (I) results in a Ag/Cu$_2$O photocatalytic material active for the reduction of CO$_2$. The photocatalytic activity of Ag/Cu$_2$O towards the CO$_2$ reduction was monitored by electrochemical methods and surface enhanced Raman spectroscopy. Our results show a beneficial effect from the deposition of Cu$_2$O onto plasmonic Ag nanostructures with respect to the overpotential necessary for CO$_2$ reduction. The electrochemical results show that the Ag/Cu$_2$O has a lower overpotential when illuminated at 633 nm and the spectroscopy results show adsorbed hydrogenated species containing C-C bond, a carboxyl group and C-H bonds. These results suggest a new material for the photoreduction of CO$_2$. 

Keywords: Electrochemistry, Material Science, Nanotechnology, Spectroelectrochemistry

Application Code: Nanotechnology

Methodology Code: Electrochemistry
There is a growing interest in the development of Surface-Enhanced Raman Scattering (SERS) substrates by tailoring nanostructured metal properties such as particle density, shape or size because they affect significantly their plasmonic properties, and, hence, the SERS effect. These substrates are typically fabricated by complex patterning techniques or with colloidal nanoparticles. Electrochemical methods have also been used for the generation of SERS-active electrodes. The activation of silver electrodes is normally carried out by oxidation-reduction cycling in chloride-based media, and experimental parameters such as other electrolytes or the presence of structure-directing agents are not typically considered. However, they could lead to the in situ generation of nanostructures with different physical (shape, size, density) and optical properties, which could enhance the SERS effect.

In this work, the electrogeneration of particular silver nanoscale features on screen-printed electrodes was carried out to obtain SERS-active surfaces. The effect of several factors such as the electrolyte, electrochemical parameters and the presence of structure-directing agents was evaluated. Scanning electron microscopy was used for the characterization of the electrogenerated nanofeatures. The experimental conditions used during the electrogeneration have a significant effect on the density, shape and size of the silver particles generated on the electrode surface, so these properties could be tuned by selecting the appropriate parameters leading to surfaces with specific functionalization. Simultaneously to the silver electrogeneration, the in situ SERS detection of some species was performed to evaluate the enhancement produced by the different nanoscale silver features at different stages of their formation.

Keywords: Electrode Surfaces, Raman Spectroscopy, Spectroelectrochemistry, Surface Enhanced Raman Spectro
Application Code: Nanotechnology
Methodology Code: Electrochemistry
Bivalve molluscs are able to accumulate metals and metalloids at different concentrations in their tissues and organs [1]. Due to the potential risks of bioaccumulation of metallic cations such as Cd(II) and Pb(II) by these marine organisms in human health, it is necessary to have analytical methodologies for their detection. In this work, a graphene-based material (electrochemically reduced graphene oxide, ErGO) was used as a modifying agent of an electrochemical sensor for trace metal detection in three species of bivalve molluscs (Venus antiqua, Mesodesma donacium and Ostrea chilensis). The modified electrode was characterized by high resolution scanning electronic microscopy (HR-SEM) observing a heterogeneous surface in terms of distribution. On the other hand, a variable electrochemical reactivity was observed by scanning electrochemical microscopy (SECM) and with Raman spectroscopy the electrochemical reduction process of graphene oxide was confirmed. The simultaneous detection of Cd(II) and Pb(II) were conducted by using square wave anodic stripping voltammetry (SWASV). The optimum conditions for GC/ErGO were as follows: 0.1 mol L-1 Acetate buffer solution with pH = 4.5, accumulation potential (Eacc) = -1.2 V and accumulation time (tacc) = 90 s. A linear range was observed from 15-105 µg L-1 with a limit of detection (LOD) of 6.86 µg L-1 for Pb(II) and 5.71 µg L-1 for Cd(II). The methodology was applied in the simultaneous determination of Cd(II) and Pb(II) in bivalve molluscs compared with ICP-MS with satisfactory results.

References


Acknowledgments

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Reconstruction of Distributions of Nanoparticles or Electroactive Nano-Components in Electrochemical Arrays Based on Chronoamperometric Data

The main scope of this work [1] was to establish and then to test a simple mathematical and numerical procedure for the reconstruction of probability density functions characterizing the distribution of electroactive or electrocatalytic of near-spherical nano-components present or deposited on a planar electrochemically-inert surface. The reconstruction procedure requires as an entry a time-dependent chronoamperometric current responses of the corresponding electrochemical array. The mathematical and numerical validity of the procedure was established for three types of arrays: one is a periodical, two others are involving random dispersions. Indeed, altogether, these three types represent most frequent surface distributions of electroactive components in electrochemical micro-/nanoarrays used for (bio)analytical or electrocatalytic (viz., using perfectly tailored nanocatalyst crystals) purposes. This work takes advantage of our recent research on regular and randomly distributed micro- and nanodisk electrodes [2, 3]. Proposed reconstruction procedure is easily implementable in the most popular commercial mathematical programs (e.g., Mathematica). Albeit the simplicity of its implementation, it allowed recovering probability density functions with an excellent precision, even when the available time-range experimentally accessible results too short for its rigorous application, being thus perfectly adequate to most experimental purposes.

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References:

Keywords: Data Analysis, Electrochemistry, Nanotechnology, Particle Size and Distribution
Application Code: Nanotechnology
Methodology Code: Electrochemistry
A high surface area nanoporous gold film (black) was prepared on a gold electrode surface by anodization followed chemical reduction method using dopamine as a reducing agent and the electrocatalytic activity of such platform was studied towards hydrogen peroxide (H2O2) reduction. Nanoporous structures have been typically fabricated by using dealloying and template methods. However, the impurities present in the porous film, requirement of corrosive chemicals and long-time associated with such procedures limit their use. Lately, we have prepared NPG films via anodization followed by electrochemical reduction and by electrodeposition via dynamic hydrogen bubble template methods, and the fabricated sensors were used for dissolved oxygen and hydrogen peroxide measurements at low overpotential. In this work, we have fabricated the NPG film (black) on a gold electrode surface by a two-steps method (i) anodization of gold at an applied potential of 2.0 V for 15 min in 0.5 mol L-1 H2SO4 solution (orange yellow color formed on the surface), (ii) chemical reduction of anodized gold using dopamine solution (10 mmol L-1, black color formed instantaneously when the anodized gold was immersed in the dopamine solution). Dopamine reduces the anodized gold and a stable black film is formed on the gold surface, whereas a dopamine-o-quinone product is generated in the solution (solution color changes into brown after the immersion). The as-fabricated NPG film displayed large surface area & roughness factor compared to the bare Au electrode. The electrocatalytic activity was explored for hydrogen peroxide (H2O2) reduction in PBS solution and excellent electrocatalytic activity in terms of reducing the overpotential and onset potential towards less negative potential with enhanced current signal (Fig.1) was observed.

Acknowledgement: FAPESP (Grant No.2014/15215-5 & 2015/20776-9), CNPq, and CAPES.

Keywords: Sensors, Voltammetry, Electrochemistry, Nanotechnology

Application Code: Other

Methodology Code: Electrochemistry
A major hurdle in the development of next-generation biosensors is the ability to detect single molecules and the use of this detection to form an appropriate assessment of the larger biological system. Once this has been achieved, it will be possible to build new and more efficient biosensors that can detect subtle concentration changes in early disease stages and biosensors that no longer require complicated calibration methods. Owing to the unique optical properties of plasmonic nanoparticles (such as gold nanoparticles), they offer the possibility to develop sensing interfaces that are capable of these ultra-low detection limits. Current spectroscopic techniques are able to sensitively analyse spectral shifts of these nanoparticles, however, these nanoparticles need to be analysed in a high-throughput manner in order to obtain a useful assessment of a biological system.

This work aims to develop a technique for sensitive and high-throughput analysis of plasmonic nanoparticles, as well as the construction of a biosensing interface capable of single molecule detection. Light scattered by the plasmonic nanoparticles is imaged using a dark-field microscope and captured using a commercially available CMOS camera. The image is then processed to identify single nanoparticles and colour information (hue) is decoded from each particle, which is then used to identify peak spectral information. We demonstrate that this technique is capable of analysing spectral information from several thousands of single nanoparticles per image. This technique offers the potential to develop a biosensor with an array of hundreds of thousands nanoparticles that are able to be analysed rapidly and sensitively. However, in order to detect at the single molecule range, it is necessary to employ a signal enhancement step. Utilising antibody sandwich assays and enzyme-mediated growth of nanoparticles, we demonstrate the ability for this biosensor to be utilised for analyte detection.

Abstract Text

Keywords: Biosensors, Microscopy, Nanotechnology, Single Molecule
Among different electrochromic materials conducting polymers and their derivatives are attractive due to their ease of processability, good stability, rapid response times and high optical properties [1]. Electrochromic properties of conducting polymers can be adopted for analytical purposes, e.g. for determination of some heavy metal ions [2]. Proper attachment of conducting polymer layer on conducting surface of electrode is critical during the formation of durable sensor [3].

In recent research we have investigated the formation of conducting polymers (polyaniline, polypyrrole) and copolymers layers on Indium Tin Oxide (ITO) covered glass slides for electrochromic gas sensors. Several different chemical and electrochemical approaches were applied in order to cover ITO glass by polymers or copolymers. The influence of layer thickness and composition on the electrochromic properties was determined. Morphological characterization of surfaces was performed by Scanning Electron Microscopy and Atomic Force Microscopy. Fourier Transform Infrared Spectroscopy was used for the identification of layers composition, additives and contaminants. The stability of polymer layers formed on ITO-covered glass during electrochemical treatment was evaluated.

Acknowledgement
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References

Keywords: Electrochemistry, Electrode Surfaces, Polymers & Plastics, Surface Analysis
Application Code: Material Science
Methodology Code: Sensors
In this paper, we report a simple one-step synthesis of well-dispersed amorphous cobalt hydroxide/oxide-modified graphene oxide (CoO$_{\text{x}}$/GO) possessing peroxidase-like catalytic activity, and its application for the detection of CN$^{-}$ ions. CoO$_{\text{x}}$/GO is formed and deposited in situ on the GO surface through the reaction between GO (size ~240 nm) and Co$^{2+}$ in basic solution at room temperature. We investigated the enzyme-mimicking activity of the CoO$_{\text{x}}$/GO nanohybrid in detail via the $\text{H}_{2}\text{O}_{2}$-mediated oxidation of Amplex Red (AR) to form fluorescent resorufin. The peroxidase-like activity of CoO$_{\text{x}}$/GO is utilized herein for the quantitation of $\text{H}_{2}\text{O}_{2}$ in a wide concentration range, from 100 nM to 100 $\mu$M. Interestingly, cyanide ions (CN$^{-}$) significantly inhibit the catalytic activity of the CoO$_{\text{x}}$/GO nanohybrid, which allows for the construction of a probe for the detection of CN$^{-}$ in water samples and laboratory wastes. We fabricated a membrane-based CoO$_{\text{x}}$/GO probe for the visual detection of CN$^{-}$ by preparing a thin film of CoO$_{\text{x}}$/GO on a positively charged and porous nylon membrane (N$^{+}$M). The CoO$_{\text{x}}$/GO/N$^{+}$M operates on the principle that CN$^{-}$ inhibits the catalytic activity of CoO$_{\text{x}}$/GO towards the $\text{H}_{2}\text{O}_{2}$-mediated oxidation of AR to form reddish resorufin on the membrane. The intensity of the red color of the membrane decreases with increasing CN$^{-}$ concentration, which can be easily observed by the naked eye at the nanomolar level. This cost-effective sensing system allows for the rapid and simple determination of the concentrations of CN$^{-}$ in complicated wastewater samples.

Keywords: Sensors
Application Code: Environmental
Methodology Code: Sensors
Significant interest in electrochromic/electrochemical gas sensing results from their numerous potential application in various areas such as environmental, industry and biomedical sciences. These sensors are attractive due to their low-cost, short response time, higher sensitivity, selectivity and stability [1]. Many classes of materials present electrochromic behaviour, but conjugated polymers attracted considerable attention due to their good environmental stability, biocompatibility and easy synthesis by electrochemical or chemical methods [2,3]. Incorporation of metallic nanoparticles into layers of conjugated polymers enhances electrochemical and optical properties, improves conductivity, robustness, stability and electrocatalytic activity of formed layers [4,5].

In this work electrochromic properties of layers formed using conjugated polymers (aniline, pyrrole) and metal nanoparticles (gold, silver) for the detection of carbon dioxide as target analyte were evaluated. Thin layers of polymer/nanoparticles were formed on indium tin oxide electrodes using electrochemical methods. Scanning electron microscopy and atomic force microscopy were used to determine the size of nanoparticles and surface morphology of formed layers. The transmission spectra of conjugated polymers/metal nanoparticle layers in the presence of analyte were recorded.

ACKNOWLEDGMENT
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References:
Mercury is recognized as a major environmental pollution issue for humans. In Amazonian countries, mercury releases into the environment may present serious toxic risks to many riparian communities. The methodology presented here is expected to facilitate data collection from statistically meaningful population in the Amazonian forest. The developed sensor is based on the use of gold nanorods (AuNRs) functionalized substrates. The UV/Vis absorbance profile of the AuNRs displays a strong surface plasmon resonance band that is sensitive to the aspect ratio (length/diameter) of the NRs. When the AuNRs 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. Mercury in water often exist in its positively charged ion form, thus reduction of the Hg(II) ion to Hg(0) is necessary for the amalgamation process to occur. Two methods of mercury reduction are presented here. The first is the use of sodium borohydride as a reducing agent combined with flow injection analysis (FIA) in order to create a constant flow of sample, thereby compounding the wavelength shift. The second method involves the use of a conductive indium-tin-oxide (ITO) substrate to create a working electrode from the ITO-AuNR substrate, allowing for mercury reduction via voltammetry. Their applicability to the on-site analysis of mercury in remote locations is discussed. The acquired knowledge is expected to benefit the development of efficient environmental remediation processes, which is extremely relevant for a globally sustainable environment.
Direct electric heating in electrochemistry, or so-called hot wire electrochemistry, has drawn lots of attention since its breakthrough in the 1990s [1, 2]. After the AC distortion problem was solved with a symmetric electrode arrangement and a symmetric inductor bridge [3], this micro-wire joule heating technology became more popular because of the advantages, such as low cost, instantaneous heating of the electrode and its nearest surroundings to the desired temperature without changing the temperature of the bulk solution, easy cleaning of micro-wire electrodes by glowing in air, etc. Although this technique offers electrochemical measurements at far above the boiling point, this has been done through pulse heating. A maximum 250 °C with 5 ms heating pulses has been reported. For various applications, continuous heating above the boiling point would be more useful. Our goal of this work is to develop a new technique for such purposes. We have modified our wire electrode fabrication method and made it more environmentally friendly by using recycled pen barrels and printed circuit board (PCB) bits (4x12x1.6 mm, less copper to be disposed and more flexible to make micro-wire electrodes with any length). Our open circuit potentiometry data have shown that reproducible temperatures above the boiling point (up to 120 °C) have been achieved for both wax-sealed and laminated gold wire electrodes with an ice bath at 1 °C. The normal temperature range for paraffin-polyethylene wax mixture insulation was up to 70 °C and for lamination film it was up to 80 °C in the literature. We have successfully used the new methodology in a kinetic study of paracetamol electrochemistry with a temperature range between 1 and 120 °C and a continuous heating time of at least 2 mins. This contribution will also employ an alternative resistive thermometer method [1] to confirm the temperature calibration and explore it further under pressurized conditions, such as supercritical conditions.

Keywords: Electrochemistry, Electrodes, Potentiometry, Temperature

Application Code: Environmental

Methodology Code: Electrochemistry
Joule-heated electrodes are used for the enhancement of electrochemical analysis. Due to heating, a temperature gradient is created near the electrode surface. The temperature pulse potentiometry method was used to perform a temperature calibration for the heated screen-printed electrode, in order to apply the desired temperature during an analysis. The applied temperature of the working electrode influences its electrical resistance and the electrochemical potential of a redox couple. Open circuit potentiometric (OCP) measurements were performed with five screen-printed gold loop electrodes (AuSPE), each time applying five automatic 50 kHz AC heat pulses provided by a ThermaLab[registered] AC generator. The resistance values at each heating pulse, were obtained by measuring the heating voltage and heating current within a circuit containing a 1 • resistor. Temperature calibrations were performed using a 5 mM equimolar solution of the redox couple ferri/ferrocyanide in 0.1 M of potassium chloride, and maintained at 20°C bulk temperature. Potential differences produced during each heat pulse were used to calculate the electrode temperature using the temperature coefficient of the redox couple (-1.6 mV/K). The temperature calibration experiments with the five AuSPEs were shown to be reproducible and precise, with an RSD for temperature of 0.24% and 4% for resistance. The average margin error of OCP temperatures were ±0.66 K at a 95% confidence level. The temperature coefficient (\( \alpha \)) of electrical resistivity was found to be 0.0025°C [sup]-1[/sup], which is 27% lower than the theoretical value for gold metal. Comparing the OCP temperature with the resistivity method, the \( \alpha T \) was about 0.94°C and 2.8% different. In this work, a quick, reproducible and accurate temperature calibration was possible for disposable AuSPE.

Supported by the Carson Carr award and the University at Albany-SUNY research start-up funds.

Keywords: Calibration, Electrochemistry, Potentiometry, Temperature
Application Code: General Interest
Methodology Code: Electrochemistry
This presentation includes our two novel fluorescent chemosensors L1 and L2 developed by reacting 1-aminopyrene and 1-pyrenemethylamine with B6 cofactor pyridoxal, respectively. The receptor L1 exhibits a rapid colorimetric response from yellow to colourless and a distinct fluorescence enhancement at 441 nm with Fe3+ due to the cleavage of imine linkage, a chemodosimeter type sensor. The optical responses from L1 in the presence of Fe3+ are highly specific, interference-free and also observed within live HeLa cells. In contrast, the receptor L2 is highly selective and sensitive towards Zn2+. Upon interaction with Zn2+, the receptor L2 showed a distinct fluorescence enhancement at 485 nm and the fluorescent colour change from blue to bluish-green. Subsequently, when the in-situ generated L2.Zn2+ complex was interacted with various anions and amino acids, the addition of H2PO4- and cysteine retrieved the fluorescence of the receptor L2 due to the demetalation of Zn2+ from the L2.Zn2+ complex. Accordingly, the receptor L2 was developed for the highly selective, specific and sensitive detection of three important bioactive analytes, i.e., Zn2+, H2PO4- and cysteine with the detection limit down to 2.09×10-7 M, 2.18×10-7 M and 1.84×10-8 M, respectively. The receptor L2 was successfully applied for the detection of intracellular Zn2+ in live HeLa cells. Additionally, the experimental evidences were complemented with the DFT results.

Keywords: Analysis, Detection, Sensors, UV-VIS Absorbance/Luminescence
Application Code: General Interest
Methodology Code: Fluorescence/Luminescence
A Novel Electrochemical Sensor Modified with Electrochemically Reduced Graphene Oxide and Aza-Crown Macrocycles for Cadmium Determination in Water

This work develops a novel electrochemical sensor for cadmium (Cd(II)) determination, in the presence of zinc (Zn(II)) and lead (Pb(II)), in water samples based in a glassy carbon electrode (GC) modified with electrochemically reduced graphene oxide (ERGO) and 1,4,7,10-Tetraoxa-13-azacyclopentadecane (A15C) employing square wave anodic stripping voltammetry technique (SWASV).

The modified electrode (GC/ERGO/A15C) was characterized by scanning electron microscopy (SEM), finding a heterogeneous surface modification (Fig. 1), and cyclic voltammetry (CV) using the redox mediator \([\text{Fe(CN)}_6^{3-}/4^-]\) (Fig.2) resulting in a faster electron transfer process and large active area for the modified electrode.

The chemical and electrochemical variables studied and optimized were: concentration of A15C, pH, frequency, accumulation potential (E\(_{\text{acc}}\)) and time (t\(_{\text{acc}}\)). Zn(II), Cd(II) and Pb(II) can be determined under optimal conditions of E\(_{\text{acc}}\) -1.5V, t\(_{\text{acc}}\) 90 s, 15 Hz frequency, A15C 5000 mg L\(^{-1}\) and pH 5.5 with good sensitivity, precision and accuracy by standard addition method.

The developed sensor presented a linear range between 10 - 100 [micro]g L\(^{-1}\) for Cd(II) and Pb(II) with a limit of detection (LOD) of 4.5 [micro]g L\(^{-1}\) and a limit of quantification (LOQ) of 15 [micro]g L\(^{-1}\) and a linear range between 20 - 100 [micro]g L\(^{-1}\) for Zn(II) with a LOD of 6.6 [micro]g L\(^{-1}\) and a LOQ of 22.1 [micro]g L\(^{-1}\).

[i]Acknowledgments
Financial support by FONDECYT under project 1140206, DICYT (USACH) and University of Santiago of Chile.[/i]

Keywords: Analysis, Chemically Modified Electrodes, Electrochemistry, Sensors
Application Code: Environmental
Methodology Code: Electrochemistry
Corrosion rate of mild-steel (MS), zinc and aluminum as well as the sulphation rate was determined during 2010-2011 under outdoor exposure in an industrial environment at Naroda (Dist. Ahmedabad) situated in Middle Gujarat, India. Monthly corrosion rate vary from 391 to 1815, 25 to 147 and 2.2 to 21.8 mg/sq.dm correspond to MS, zinc and aluminium respectively, whereas the yearly corrosion rate vary from 7607 to 18679, 301 to 621 and 19.8 to 46.1 mg/sq.dm for mild-steel, zinc and aluminium respectively. Monthly corrosion rate was found in the decreasing order: mild steel < zinc < aluminium. Corrosion rate of these three metals was found more in rainy seasons than the rate of winter and summer season. There is a considerable loss of corrosion rate during exposure for one year of mild steel. Monthly corrosion rate of mild steel indicate a close correlation ship with rainfall \(r = 0.75\), number of rainy days \(r = 0.92\) and satisfactory correlation with sulphation rate \(r = 0.43\). No correlation appeared to exist between temperature and corrosion rate of mild, zinc and aluminium. The X-ray diffraction analysis of mild-steel corrosion product was also done.

Keywords: Electrochemistry, Electrodes, Environmental
Application Code: Environmental
Methodology Code: Electrochemistry
**Abstract Text**

Typically working electrodes used in cyclic voltammetric studies are selected because they can serve as either a source or sink of charge in the supporting electrolyte being used. Occasionally the working electrode participates in some other way that is not visible from the current-voltage curve. This would be the case when the working electrode is oxidized or reduced simultaneously with oxidation or reduction of electroactive species present in the supporting electrolyte. Here we consider the case of a gold working electrode in potassium bromide or potassium iodide in a phosphate pH 7 buffer as a supporting electrolyte. We use the EQCM technique to demonstrate that at positive potentials where the anodic current due to oxidation of the halide ion becomes controlling, the gold working electrode does more than facilitating the charge transfer. The frequency voltage curves show that gold electrode loses weight in the anodic current region. We ascribe this mass loss to be the result of formation of soluble gold halide complexes in the potential region where halide ion is oxidized to either iodine or bromine. The mass loss between potential cycles is greater in bromide containing electrolyte than iodide. In a given halide solution, the mass loss was identical during successive potential cycles and depended upon the concentration of halide.

**Keywords:** Electrochemistry, Electrodes, Sensors, Voltammetry

**Application Code:** General Interest

**Methodology Code:** Electrochemistry
Porous silicon (pSi) has been studied for many applications, including chemical sensing. A key to success in any sensing platform is understanding the analyte-pSi interactions and being able to selectively control them. Commonly, one uses the intrinsic pSi photoluminescence (PL) as the signal transduction method to sense gaseous analytes. Sensing platforms have been developed by using as prepared, H-passivated and oxidized pSi (ap-, ox-pSi). Although PL-based measurements have proven valuable for sensor development, they do not provide details into the mechanism of interaction between the Si nanocrystallites and the analyte. To rectify this problem my research has centered on exploiting Fourier transform infrared (FTIR) and Raman spectroscopy to elucidate how gaseous analytes affect the pSi surface chemistries (i.e. Si-O-Si, SiHx (x = 1-3) and OySiH (y = 1,2) band amplitudes and positions), the amorphous and nanocrystalline Si phonon band amplitudes and positions. The results from these experiments reveal the relationship between the analyte-dependent PL, IR, and Raman responses and shed light on the PL-based analytical signal origin.
Carbon based functional materials have emerged as very promising electrocatalysts in the broad domain of electrochemical sensors. We report here the electrocatalytic properties of graphene oxide (GO)/Cobalt Phthalocyanine octa-carboxylate (CoOCPc) composites (Fig. 1a) towards oxygen reduction. Metallophthalocyanines have shown excellent electrocatalytic activity in different redox processes [1], but their activity is often depressed by poor conductivity and aggregation. One of the innovative ways to mitigate these limitations and fasten the electron transfer process is to immobilize such molecules on the surface of conducting materials like graphene oxide. The immobilization of CoOCPc between layers of GO was achieved through non-covalent functionalization approach. The resulted composite was drop casted on a 3 mm glassy carbon electrode (GCE) and tested for oxygen reduction reaction (ORR) in a 3-electrode cell configuration. The linear sweep voltammograms (LSV) recorded in an oxygen saturated 0.1M KNO₃ solution are presented in Fig. 1b. for bare, GO, CoOCPc and the composite modified GCE. All modifications result in shifting the oxygen reduction overpotential towards less negative values, but the maximum shift of 0.45V can be noticed by using the composite modified GCE. Such behavior reveals a fast electron transfer kinetics because of the presence of a high number of electrocatalytically active defects in the composite layer. Moreover, an enhancement in peak current as compared to CoOCPc is observed, which results from the increased surface area of graphene oxide, facilitating a higher number of oxygen molecules to interact with the electrode. Detailed characterizations of the composite are in progress to correlate its nanostructure with superior electrochemical performance.

Acknowledgements
The authors thank FAPESP, CNPq and CAPES for the financial support.

References

Keywords: Electrochemistry, Material Science, Nanotechnology, Sensors
Application Code: Other
Methodology Code: Electrochemistry
Many organic compounds like nitrophenol and nitrobenzene are extensively used in chemical industry for manufacture of pesticides, synthetic and pharmaceutical dyes which are unpleasant and toxic in nature. Removal and decomposition of such compounds from natural and waste water is very essential. Here, electrochemical technique was devised to decompose these organic pollutants. Nitrobenzene and nitrophenol, organic pollutants, were undergone electrochemical reduction through modified graphite electrode. To enhance the interaction between electrode and pollutant, surface of the electrodes has been modified by surfactants. For this purpose sodium dodecyl sulphate has been physically incorporated at the surface of the graphite electrodes. Modified surface of the electrodes has been characterized by UV-Visible and FTIR spectroscopic techniques. Analysis and comparison of the response of the simple and modified graphite electrodes encourage the modification of electrodes for decomposition of such organic pollutants to make water free from such inhibitory and noxious chemicals.
Herein, the photoreduction of graphene oxide (GO) with titanium dioxide (TiO2) was exploited to fabricate a UV sensor for monitoring sun exposure. The output of this sensor is a decrease in the resistance of the sensor as a result of conversion of GO to reduce GO (rGO). The sensor was fabricated by simultaneous deposition of GO sheets and TiO2 nanoparticles onto interdigitated electrodes using AC electrophoresis deposition method. Changes in the resistance of the GO-TiO2 nanocomposite during repeated cycles of exposure to UV was measured to understand the sensitivity of this sensor to the UV radiation. Current-time (I-t) traces revealed that the fabricated UV sensor retains a memory of each cycle of exposure to UV, regardless of being exposed to the UV for one long cycle or several small cycles.

**Keywords:** Semiconductor, Sensors  
**Application Code:** Safety  
**Methodology Code:** Sensors
The 2D IR spectroscopy technique is introduced. The pulse sequence is described, and a very simple example is used to illustrate the nature of the 2D IR spectrum. 2D IR Chemical Exchange Spectroscopy is presented to show how the time dependence of the 2D spectrum can be employed to obtain dynamical information about molecular systems under thermal equilibrium conditions. Examples are presented. i.e., organic molecular complexes, isomerization around a carbon-carbon single bond, and protein substate interconversion. The application of 2D IR spectroscopy to obtain liquid structural dynamics through the measurement of spectral diffusion is described. Measurements of water hydrogen bond dynamics are explicated. A 2D IR pulse shaping spectrometer is introduced. It is shown that using phase cycling, highly scattering samples can be investigated with 2D IR spectroscopy. Experiments on carbon dioxide in Supported Ionic Liquid Membranes are used as an example. Finally, a new method that greatly enhances the signal from a monolayer or very thin film (~100 nm) is discussed and applied to the dynamics of a Langmuir monolayer at the air/water interface.
We have developed a new method to produce materials and liquids in extreme states of high pressures and high temperatures using a pulsed laser to launch a thin metal foil into the sample. The km/s impacts with these laser-launched flyer plates generate powerful shock waves, whose effects can be probed with emission, absorption or vibrational spectroscopies. Typical impact velocities are 1 or more km/s, producing pressures of several GPa (1 GPa = 10,000 bar) and temperatures up to 6000K. In this talk I will describe this new method and then discuss a few recent applications: probing shocks with quantum dot emission, shock compression and attenuation by metal-oxide frameworks (MOFs) and shock initiation of explosives.

Keywords: Fluorescence, High Temperature, Microscopy, Ultra Fast Spectroscopy
Application Code: Material Science
Methodology Code: Physical Measurements
Ultrafast vibrational spectroscopy in the mid-infrared spectral range provides the opportunity to probe the dynamics of electronic states involved in all stages of the singlet fission reaction through their unique vibrational frequencies. We demonstrate this capability using a model singlet fission chromophore, 6,13-bis(triisopropylsilylethynyl) pentacene (TIPS-Pn). The alkyne groups of the TIPS side chains are coupled to the conjugated framework of the pentacene cores, enabling us to directly examine the dynamics of triplet excitons that have successfully separated from interacting correlated triplet pair states in crystalline films of TIPS-Pn. Following their dissociation, triplet excitons undergo bimolecular annihilation resulting in the formation of hot ground state molecules that also exhibit unique vibrational frequencies. Because all organic molecules possess native vibrational modes, ultrafast vibrational spectroscopy offers a general approach to examine the dynamics of electronic intermediates that may inform on-going efforts to utilize singlet fission to overcome thermalization losses in photovoltaic applications.
Proteins populate a multitude of states that interconvert on a broad range of timescales. To uncover how such dynamics contribute to protein function requires facing the experimental challenges associated with both the spatial complexity of proteins and the rapid timescales of potentially important motions. To overcome these issues, we combine methods of linear and two-dimensional infrared spectroscopy, with their inherent high temporal and spatial resolution, with chemical biology approaches for specific incorporation of vibrational reporter groups into proteins that provide frequency-resolved absorptions for characterizing their local environments. We are applying this approach to measure residue-specific side chain dynamics toward evaluating their role in protein molecular recognition. This presentation will focus on our recent studies of the molecular recognition of Src homology 3 domains with proline-rich motifs and plastocyanin with cytochrome f.

Keywords: Biospectroscopy, Infrared and Raman, Protein, Vibrational Spectroscopy
Application Code: Bioanalytical
Methodology Code: Vibrational Spectroscopy
The Pittsburgh Spectroscopy Award

Terahertz EPR, Terahertz Rotational Spectroscopy, and 2D Terahertz Spectroscopy

With simple commercial sources of short-duration terahertz-frequency light pulses and inexpensive commercial spectrometers based on them now readily available, several previously challenging terahertz (THz) spectroscopies have become widely accessible. Two types of THz spectroscopy will be emphasized: THz EPR measurements of high-spin transition metal and rare earth compounds, in which zero-field splittings are determined, and THz gas-phase molecular rotational spectroscopy. In the most common configuration, a single-cycle THz pulse whose frequency content typically includes the 0.1-5 THz range (and may extend to 10 THz or more) irradiates the sample and a time-dependent free-induction decay signal is measured. Fourier transformation yields the absorption spectrum. The frequency range includes zero-field splittings of many compounds and rotational lines of small molecules.

Two-dimensional (2D) THz EPR and rotational spectroscopies have also been demonstrated, using stronger THz fields that are generated routinely (though at higher cost) in many labs. These are among a wide range of nonlinear THz spectroscopies that can reveal molecular information not obtainable through linear spectroscopy measurements. With strong THz fields, considerable control over molecular and material responses becomes possible. Some recent results including THz-induced electroluminescence and colossal Stark shifts in quantum dots, THz-induced chemical decomposition, and THz-induced phase transitions will be reviewed briefly. Possibilities for THz-driven orientation of molecular dipoles and magnetic domains will be discussed.

**Keywords:** Luminescence, Magnetic Resonance, Molecular Spectroscopy, Spectroscopy

**Application Code:** General Interest

**Methodology Code:** Molecular Spectroscopy
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 derivatized cyclofructan 6 exhibits tremendous enatioselectivity toward chiral primary amines. When evaluated with 120 primary amine containing racemates, the isopropyl derivatized cyclofructan 6 phase showed a 93% success rate. This type of “class” selectivity allows researchers intuitive column selection and bypasses extraneous time spent on column screening. Conversely, aromatic derivatized cyclofructans prove to be broadly selective for a wide range of types of racemates. The evaluation of more 20 cyclofructan derivatives allowed for the identification of three that are most successful (one aliphatic and two aromatic). These phases have been used in a variety of applications, such as the separation of chiral agrochemicals, illicit drugs, controlled substances, ethano-bridged troger bases, pyrrole-imidazole alkaloids, and metal complexes which exhibit axial chirality. Lastly, although native cyclofructan is a poor chiral selector, it is an exceptional HILIC stationary phase. The use of native cyclofructan as a HILIC stationary phase produces better separations for certain classes of polar compounds compared to the few existing commercial HILIC phases. Recently, cyclofructan based chiral and HILIC selectors have been bonded to superficially porous particles and UHPLC silica to produce high-throughput and highly efficient separations.

Disclosure: Zachary S. Breitbach is a current employee of AbbVie Inc. Data present employment.

Keywords: Chiral Separations, Chromatography, HPLC, HPLC Columns
Application Code: General Interest
Methodology Code: Liquid Chromatography
Abstract Text

Enantiomeric separations are a fundamental part of many biological and pharmacological studies. They also have important ramifications in regulatory actions, forensic science, exobiology, sports doping and natural product discovery; among other areas. New and improved chiral methods are becoming essential for many of these studies as they provide greater selectivity, enhanced efficiency and analysis speed. Examples will be given on the role of chiral separations in many of these areas. For example D-amino acid studies in biological systems that took over a year to complete can now be done in a week. The impact of these studies will be described. A new column for the separation of nicotine enantiomers and analogues allow facile evaluation of synthetic non-tobacco derived products and raises regulatory questions. Chiral separations were used for natural products and correcting their literature optical rotations. These and other interesting uses of state-of-the-art enantiomeric separations will be presented.

Keywords: Bioanalytical, Chiral, Chiral Separations, Pharmaceutical
Application Code: Bioanalytical
Methodology Code: Separation Sciences
A synopsis of our recently presented review by this same title in Trends in Analytical Chemistry, now available online, is presented. We present a survey of different strategies for chromatographic method development in pharmaceutical research and development, with particular emphasis on how the field has evolved over time. Owing to the widespread utilization of chromatography within diverse areas of pharmaceutical research, a variety of strategies for method development have arisen. We survey the current state of the art, discuss recent trends and approaches and highlight future prospects and capability gaps.

Keywords: Chromatography, Method Development, Pharmaceutical
Application Code: Pharmaceutical
Methodology Code: Liquid Chromatography
Magnetic Ionic Liquids as PCR Compatible Solvents for Rapid Nucleic Acid Extraction and Analysis

Rapid nucleic acid analysis has become a vital component for clinical diagnostics, food safety, genomics, and microbiology. Techniques such as PCR and DNA sequencing are capable of detecting very small quantities of nucleic acids but are also susceptible to interfering constituents within complex samples. Therefore, the isolation of sufficiently pure nucleic acids represents a formidable bottleneck in DNA analysis. This talk will discuss the progress my group has made in the development of hydrophobic magnetic ionic liquid (MIL) solvents that are capable of rapidly extracting DNA and mRNA from biological samples. The vast structural versatility of MILs permits the design of PCR buffers that enable the amplification of target genes in a nucleic acid extracted from crude bacterial cell lysate.

Keywords: Bioanalytical, Nucleic Acids, Sample Preparation, Separation Sciences
Application Code: Bioanalytical
Methodology Code: Sampling and Sample Preparation
Gas Chromatography-Vacuum Ultraviolet (GC/VUV) Spectroscopy is a relatively new technique in an otherwise mature field of GC. GC-VUV allows for full spectrum acquisition from 125 – 240 nm at rates as high as 90 Hz. Judicious choice of spectral filters during data processing allows for signal-to-noise optimization and qualitative distinction of classes of compounds. The additive nature of Beers Law allows for the deconvolution of co-eluting compounds, even isomers, when spectra are distinct. Here we present the role of GC-VUV in the quality control (QC) of three industries.

The analysis of component and final product gasoline streams for Paraffins, Isoparaffins, Olefins, Naphthenes and Aromatics (PIONA) is an important QC parameter to monitor fuel performance and efficiency. Here we demonstrate the usefulness of GC-VUV Time Interval Deconvolution (TID), ASTM D8071, in competing with other standard methods.

The analysis of trans- fats in food products is an important QC parameter in food safety. Standard GC methods require a highly polar cyanopropyl phase to efficiently separate cis- and trans- fatty acid isomers after their derivatization to methyl esters. Here we demonstrate the usefulness of GC-VUV in identifying cis- and trans- isomers as well as distinguishing certain positional isomers via their unique absorbance spectra.

Residual solvents are regulated to various levels, according to their toxicity, in pharmaceutical products. Here we demonstrate the capabilities of static headspace GC-VUV for residual solvents analysis. Rapid analysis of Class 1 (e.g., benzene and carbon tetrachloride) and Class 2 solvents in model excipients was aided by the spectral deconvolution of coeluting solvents.

**Keywords:** Food Safety, Fuels\Energy\Petrochemical, Pharmaceutical, UV-VIS Absorbance/Luminescence

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

**Methodology Code:** Gas Chromatography
While 11 disinfection by-products (DBPs) are currently regulated in the U.S., more than 600 DBPs have been reported in scientific literature, many of which are more toxic than those regulated. Therefore, there are new efforts to try to minimize the formation of these unregulated, priority DBPs in drinking water. Of the removal strategies, granular activated carbon (GAC), has received renewed interest, due to its ability to remove natural organic matter (NOM) precursors to DBPs. However, while GAC can reduce the formation of many regulated DBPs, there was indication that brominated species may actually increase in formation. This is of concern because bromine-containing DBPs are generally much more toxic than chlorine-containing DBPs. As a result, there is a question of whether the drinking water would actually be safer with the use of GAC. Thus, we embarked on a study to investigate the ability of GAC to remove ~60 priority, unregulated DBPs, many of which contain bromine in their structures. Because iodine-containing DBPs are typically more toxic than brominated DBPs, these were also investigated. New gas chromatography (GC)-mass spectrometry (MS) methods were created to measure these priority DBPs at ng/L detection limits. Total organic chlorine, bromine, and iodine (TOCl, TOBr, and TOI) were also measured. In addition, the impact of the age of the GAC, types of GAC, temperature, impacts of wastewater, and prechlorination before GAC, were investigated. Results show promise for the use of GAC, with the exception of a few priority, unregulated brominated DBPs that increased in formation.
Chlorination is one of the most widely used techniques for biofouling control in large industrial units (nuclear or thermal power plants, petrochemical and steel industries, desalination units, ...) and also for the control and management of ships ballast water before discharge into the sea. The major concern related to the use of this process is, to some extent, dumping of chlorine in the environment (typically at doses varying between 0.5–1.5 mg L^-1) with known impacts on fauna and flora but above all, potential output of many halogenated compounds formed by complex reactions between the residual chlorine, and inorganic and organic constituents, natural and man present in the receiving environment. Because of discharges in seawater, the nature of chlorination by-products (CBPs) generated is highly shifted to brominated compounds, and to a lesser extent, due to the presence of iodine, to iodinated compounds.

This presentation will highlight the importance of identifying the nature of compounds and not merely referring to the global parameter Total Residual Oxidant (TRO), as often mentioned in national or local regulations. This is especially crucial considering the high toxicity of brominated and iodinated compounds, even at low concentrations. It will also focus on the difficulties arising from analysing low levels of these compounds in saline matrices and will present new advances in analytical methods and sample preparations to overcome these problems. Analytical procedures applied to real environmental situations will be used for illustration purposes (with examples taken from literature and from our own results), and will allow us to present use of several techniques (such as EOX/AOX, GC-ECD, GC-MS, LC-MS-MS).

Keywords: Analysis, Contamination, Environmental/Water
Application Code: Environmental
Methodology Code: Liquid Chromatography/Mass Spectrometry
Chlorine in swimming pool water is required to ensure a good microbiological quality and thus the safety of swimming pool users. However chlorine reacts with several compounds released by bathers to form disinfection by-products (DBPs). Due to the harmful effects of some DBPs a particular attention must be paid on these compounds (THMs and NCl3 for example). The volatile fraction of the DBPs is transferred in ambient air while less volatile DBPs remain in water. Monitoring DBPs in both water and air using conventional approach require the use of multiple techniques and sample preparation strategies (DPD-spectrophotometric assay for chloramines in water, ion chromatographic measurements for trichloramine after reduction in chloride ion and gas chromatographic analysis for the trihalomethanes with headspace and carbotrap for water and air, respectively). Moreover, the low stability of several compounds and the lack of specificity of some methods make difficult to measure DBPs in water and atmosphere pool.

The MIMS technique (Membrane Introduction Mass Spectrometry) provides a solution to these problems by in-situ measurement of DBPs with the specificity and sensitivity of mass spectrometer instrument. MIMS is based on the simultaneous permeation of the selected compounds from the samples of air or water through a PDMS membrane. DBPs were then identified and quantified with a quadrupole analyzer after electron ionization. This study focuses on the effect of analytical conditions on the determination of DBPs (pH, ionic strength, temperature, hydraulics, membrane size...). Results show that MIMS is a promising technology for the simultaneous quantification of volatile DBP in both water and air. However the measurement of THMs and chloramines is affected by some operating conditions and requires the monitoring of several ions and mathematical corrections of the signal.

Abstract Text

Chlorine in swimming pool water is required to ensure a good microbiological quality and thus the safety of swimming pool users. However chlorine reacts with several compounds released by bathers to form disinfection by-products (DBPs). Due to the harmful effects of some DBPs a particular attention must be paid on these compounds (THMs and NCl3 for example). The volatile fraction of the DBPs is transferred in ambient air while less volatile DBPs remain in water. Monitoring DBPs in both water and air using conventional approach require the use of multiple techniques and sample preparation strategies (DPD-spectrophotometric assay for chloramines in water, ion chromatographic measurements for trichloramine after reduction in chloride ion and gas chromatographic analysis for the trihalomethanes with headspace and carbotrap for water and air, respectively). Moreover, the low stability of several compounds and the lack of specificity of some methods make difficult to measure DBPs in water and atmosphere pool.

The MIMS technique (Membrane Introduction Mass Spectrometry) provides a solution to these problems by in-situ measurement of DBPs with the specificity and sensitivity of mass spectrometer instrument. MIMS is based on the simultaneous permeation of the selected compounds from the samples of air or water through a PDMS membrane. DBPs were then identified and quantified with a quadrupole analyzer after electron ionization. This study focuses on the effect of analytical conditions on the determination of DBPs (pH, ionic strength, temperature, hydraulics, membrane size...). Results show that MIMS is a promising technology for the simultaneous quantification of volatile DBP in both water and air. However the measurement of THMs and chloramines is affected by some operating conditions and requires the monitoring of several ions and mathematical corrections of the signal.

Keywords: Environmental/Air, Environmental/Water, Mass Spectrometry
Application Code: Environmental
Methodology Code: Mass Spectrometry
Disinfection to kill pathogens in drinking water was one of the most important public health achievements of the past century. As a result, food may now surpass drinking water as a pathogen exposure route in the U.S. Much like drinking water disinfection, guidelines will need to be developed specifying disinfectant types, doses and contact times for produce washing. Following the discovery that disinfectant reactions with dissolved organic precursors in water supplies can produce potentially carcinogenic disinfection byproducts (DBPs), drinking water facilities have altered disinfectant strategies to achieve a balance between the acute risk posed by pathogens and the chronic risk posed by DBPs. As guidelines are developed for produce disinfection, it will be important to balance these risks. Significant DBP formation from produce disinfection may be expected based upon the high chlorine doses and high precursor concentrations (e.g., ~80 g carbon/L lettuce) relative to the lower chlorine doses (~5 mg/L as Cl2) and precursor concentrations (~2 mg/L dissolved organic carbon (DOC)) encountered in drinking water. We previously demonstrated the conversion of tyrosine residues in model proteins to 3-chlorotyrosine and 3,5-dichlorotyrosine at molar yields up to 50% upon treatment with chlorine. If these non-volatile DBPs are formed from proteins during chlorine disinfection of produce, they are expected to persist until consumption by consumers, and to be bioavailable, since the human digestive system would liberate these chlorinated tyrosines from proteins. This study developed a LC-MS-based method to extract and quantify 3-chlorotyrosine and 3,5-dichlorotyrosine formed during chlorine disinfection of lettuce and spinach. The levels of these DBPs were compared to those of the volatile DBPs targeted in drinking water research in the spent washwater (e.g., trihalomethanes). We also demonstrated the detection of these DBPs in store-bought bagged lettuce and spinach.

Keywords: Amino Acids, Mass Spectrometry
Application Code: Food Safety
Methodology Code: Liquid Chromatography/Mass Spectrometry
Disinfection of swimming pools is critical to prevent outbreaks of infectious diseases in swimming pools. Disinfectants react with organic compounds introduced into pools by swimmers leading to the formation of disinfection by-products (DBPs). Little is known about the contribution of different anthropogenic organic precursors to the formation of DBPs in chlorinated seawater swimming pools. The present study investigated the occurrence of DBPs in chlorinated seawater pools, assessed their formation from different precursors, and examined the genotoxicity of two compounds. Pool water samples were obtained from seawater swimming pools located in Southeastern France. Occurrence of DBPs was investigated using gas chromatography coupled to electron capture detector (GC-ECD) and mass spectrometry (GC-MS). Extractable organic halide (EOX) was also measured using a combustion/micro-coulometer system. Laboratory-controlled chlorination experiments were conducted in reconstituted seawater to study the reactions between chlorine and different organic precursors released by swimmers such as sunscreens, body fluids and hair. Analysis of DBP formation was performed using GC-ECD, GC-MS and liquid chromatography (LC) coupled to high-resolution MS. The genotoxicity of bromal hydrate (BH), which was detected in chlorination experiments as well as in field samples of seawater swimming pools, was compared to that of its chlorinated analogue chloral hydrate (CH) using a battery of genotoxicity tests. Overall, this study showed that the predominant DBPs detected in the seawater pools were dibromoacetic acid, tribromoacetic acid, bromoform and dibromoacetonitrile (DBAN). Body fluids and hair contributed to the formation of DBAN and bromoform. UV filters contributed to the formation of brominated transformation products and bromoform. Based on the identified byproducts, transformation pathways of UV filters were proposed. BH exhibited higher genotoxic activity in comparison to CH.

Keywords: Gas Chromatography/Mass Spectrometry, Identification, Liquid Chromatography/Mass Spectroscopy
Application Code: Environmental
Methodology Code: Mass Spectrometry
Multimodal Chemical Imaging Using Laser Capture Microdissection (LMD)-Liquid Vortex Capture (LVC) Mass Spectrometry

An approach to high spatial resolution ambient sampling/ionization coupling a commercial laser microdissection (LMD) system with electrospray ionization (ESI)-MS via a liquid vortex capture (LVC) probe is presented. Applications on tissue will be presented highlighting the co-registered bright-field, fluorescence, and mass spectrometry imaging capabilities of the system. Absolute quantitation is demonstrated for 40 µm x 40 µm and 20 µm x 20 µm sampling sizes of tissue through ‘cut and drop’ sampling and a conventional, simple to implement solution-based, isotopically labeled internal standard strategy. Advancements to the design and operation of the LVC-MS probe with an emphasis on quantitative analysis and control of particulate capture will be discussed.

A Leica LMD7000 integrated with an AB Sciex 5500 or 5600+ mass spectrometer using a LVC probe was used for all experiments. Recently laser capture microdissection-liquid vortex capture/electrospray ionization mass spectrometry (LMD-LVC/ESI-MS) demonstrated the ability to quantify low levels of dosed drug in thin tissue microdissections (20-40 µm) using laser ‘cut and drop’ sampling (CnD). This sampling mode provided 100% capture of material versus laser ablation (LA) sampling where capture efficiency (CEff) was ~50%. To quantify using LA, CEff must be improved to 100% or at least remain a constant value regardless of the sample environment, analyte composition, and laser parameters. If CEff is a constant, it would enable rapid quantitative MS imaging with high spatial resolution. Using a series of analytes selected for their chemical and physical heterogeneity and their respective internal standards, collection efficiencies and ratios between signals from LA-LVC sampling were found to vary significantly depending on the analyte and sampling condition making quantitative analysis by laser ablation non-trivial. The dynamics of the LVC-probe in relation to these findings are discussed.

Keywords: Bioanalytical, Imaging, Instrumentation, Mass Spectrometry
Application Code: Bioanalytical
Methodology Code: Mass Spectrometry
Advances Multimodal Chemical Imaging Instrumentation

Nonlinear Optical Analysis of Pharmaceutical Materials

Second harmonic generation (SHG) microscopy is highly selective to crystals of homochiral molecules, providing a means for quantitative and qualitative analysis of active pharmaceutical ingredients throughout the formulations pipeline. Once a potential therapeutic small molecule is identified, experiments to map the set of available crystalline forms typically precedes in vivo screenings. Polarization-dependent SHG microscopy enables rapid and selective identification of outliers on nanogram scale quantities, compatible with high-throughput screening platforms. Using SHG to guide targeted analysis by Raman and synchrotron X-ray diffraction provided independent confirmation of initial assessments of crystal form by polarization-dependent analysis at parts per million levels. Following identification of crystallization-space, active pharmaceutical ingredients (APIs) with poor aqueous solubility are often cast within amorphous solid dispersions (ASDs) to prevent crystallization. SHG microscopy enables characterization of nucleation and growth kinetics with limits of detection orders of magnitude below alternative conventional benchtop methods in accelerated stability studies designed to assess ASD formulations. SHG can also inform subsequent dissolution tests, enabling measurements of crystallization kinetics directly within turbid media designed to replicate realistic conditions for oral administration. These collective capabilities have the potential to significantly reduce the timelines required for delivery of therapeutics capable of saving or improving quality of life.

Keywords: Chiral, Microscopy, Pharmaceutical, Ultra Fast Spectroscopy

Application Code: Pharmaceutical
Methodology Code: Microscopy
Exploration of chemical heterogeneity is important for understanding normal and pathological functioning of the brain. Recent progress in the development of chemical characterization approaches has led to improved coverage of the spatio-chemical complexity of the brain. We combine mass spectrometry imaging (MSI) and vibrational spectroscopic imaging (Fourier transform infrared spectroscopy imaging, and stimulated Raman scattering microscopy). These technologies are complimentary, label-free and multiplexed, allowing detection of tens to hundreds of individual analytes and functional groups from dispersed cells and tissue slices with up to sub-micron spatial resolution and zeptomole detection limits.

We have successfully interfaced these measurements to work on the same samples with rat brain slices or single brain cells. Both IR and Raman imaging revealed diverse spatial distributions of lipids and proteins. Information content of vibrational spectroscopic imaging was enhanced with highly detailed MS examination of brain slices performed with MSI and individual cells analyzed using high-throughput single cell MS. A number of metabolite signals, including physiologically important lipid species, were observed. Identities of many detected signals were determined using ultrahigh mass resolution FT-MS and FT-MS/MS measurements.

Significant attention was devoted to development and application of computational methods to allow chemical imaging data co-registration, correlation, and statistical analysis. Our postprocessing steps allow chemical image tile stitching and removal of artifacts, as well as registration of data obtained by complimentary analytical methods. Different methods of multivariate analysis, such as PCA and t-SNE were successfully adapted for multimodal data analysis. This work constitutes a new toolset capable of profiling the mammalian brain with unparalleled chemical and spatial detail and categorize cells based on their chemical profiles.

**Keywords:** Imaging, Mass Spectrometry, Neurochemistry, Vibrational Spectroscopy

**Application Code:** Neurochemistry

**Methodology Code:** Mass Spectrometry
The world of the Analytical Scientist continues to evolve into one which is filled with complexity as spatial regimes contract into the nanoworld and concentrations cover the range of bulk/major to extreme trace constituents/contaminants. Ultimately the perennial questions of “What is it?” “About how much is there?” and of course, “Exactly how much is there?” resonates with anyone doing analyses be it mineral samples from mining operations, gunk from a petroleum refinery or spent nuclear fuel. When spectroscopic methods involve imaging where characteristic spectroscopic features are presented as elemental distribution, molecular functional groups or buried structural features, a more comprehensive understanding of the material can be realized. If these spectroscopic imaging techniques are orthogonal such as elemental and molecular, a quantum level increase in chemical information is achieved when such spectroscopic imaging is integrated to result in a “1+1=3” increase in information content. This presentation will highlight the integration of elemental imaging based on micro X-ray fluorescence at spatial scales from millimeters to micrometers along with molecular imaging using Raman and infrared at the micrometer scale as well as structural details using computed tomography at the micrometer scale. Spectroscopic imaging integration involves hyperspectral imaging where large data cubes which contain spectroscopic information in two-dimensional spatial locations which ultimately requires further data processing to produce chemically relevant information. Examples will be presented ranging from simple image correlations of elemental images from micro X-ray fluorescence mapping with micro Raman and micro IR molecular images to preliminary examination of chemometric processing to extract the chemical nature of the information present.

Keywords: Elemental Analysis, FTIR, Materials Characterization, X-ray Fluorescence
Application Code: Material Science
Methodology Code: X-ray Techniques
Advances Multimodal Chemical Imaging Instrumentation

Multimodal Spectroscopic Imaging - The Whole is Greater Than the Sum of Its Parts

The realization of label-free molecule specific imaging of tissue is crucial for many envisioned applications in medicine, e.g. non-invasive histopathologic examination of tissue. Thus, new approaches for a fast and reliable in vivo or near in vivo tissue characterization is needed. In this context, spectroscopic approaches are especially noteworthy. This presentation reports about the spectroscopic detection of tissue pathologies, focusing on the determination of the tumor type and grade and a better delineation of tumor margins. It will be shown that the combination of different spectroscopic techniques in a multimodal imaging approach is very beneficial to meet the aforementioned challenges. Two major approaches are presented: First, multi-contrast imaging utilizing imaging approaches with similar image acquisition times. Here, modalities requiring similar experimental equipment are combined such as the integration of various nonlinear techniques like e.g. the combination of CARS (coherent anti-Stokes Raman spectroscopy) or SRS (stimulated Raman spectroscopy) with two-photon excited autofluorescence (TPEF), second harmonic generation (SHG), fluorescence life-time imaging microscopy (FLIM) etc. Second, fast imaging techniques (e.g. FLIM) are combined with slow but specific approaches like linear Raman spectroscopy to link a large field of view of morphological information with a richness of molecular detail of selected points or confined areas.

In summary, we will present various combinations of spectroscopic modalities to maximize the extractable information to obtain a reliable diagnosis. The presented examples highlight the potential of multi-contrast spectroscopy to solve challenges currently faced by clinical pathology.

Acknowledgment
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Keywords: Biomedical, Chemometrics, Molecular Spectroscopy, Raman Spectroscopy

Application Code: Biomedical

Methodology Code: Molecular Spectroscopy
## Biochemical Analysis at the Limits of Single Molecules and Single Cells

### In Vivo Electrochemical Monitoring of Ascorbate

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

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

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

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

4) The kinetics of the exocytosis of endogenous ascorbate from a single adrenal chromaffin cell would be presented.

### Abstract Text

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### Keywords:
- Biosensors, Monitoring, On-line
- Bioanalytical
- Electrochemistry
Biochemical Analysis at the Limits of Single Molecules and Single Cells

**Abstract Title**

Single-Cell Proteomic Analysis of Circulating Hematopoietic Stem/Progenitor Cells in Patients with Myeloproliferative Diseases

**Primary Author**

Rong Fan  
Yale University

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**Abstract Text**

Although the idea of using circulating tumor cells for early detection of tumor progression has been widely adopted in a number of clinical trials, it was not considered a unique approach for hematologic disorders because mutant/cancerous cells already circulate in blood stream and the cytopathologic testing of these cells have been routinely conducted for diagnosis. However, we realized that the majority of these cells in blood do not represent mechanistically the cells of origin that cause disease development and progression. It is a fraction of hematopoietic stem/progenitor cells (HSPC) in bone marrow that harbored oncogenic mutations and undergo clonal expansion to give rise to a wide range of hematologic malignancies. Interestingly, HSPCs are shed from bone marrow (BM) to blood in patients. It is their functions, which cannot be readily characterized by mutational profiles, that mediate the mechanism of disease progression and therapeutic response. Herein we developed a single-cell protein secretion profiling technology to measure the function of circulating HPSCs (~1000 per mL) in patients with myeloproliferative neoplasm (MPN). It is a microchip platform that allows for highly multiplexed (up to 42) cytokine production measurement in single cells. It was applied to a prospective cohort of ~50 patients for detecting abnormal cytokine-secreting hematopoietic cells in blood. The results revealed the potential to discover new biomarkers based on single-cell protein signatures for early diagnosis of disease progression. Two new biomarkers we identified demonstrated the ability to detect fibrotic progression of patients from non-fibrotic stages (P value < 0.01) as well as stratify patients with closely related conditions for precision medicine.

**Keywords:** Biomedical, Immunoassay, Proteomics

**Application Code:** Biomedical

**Methodology Code:** Microfluidics/Lab-on-a-Chip
The measurement of simultaneous parameters in a single cell is important because useful information can be collected from individual cells to help understanding the disease manifests or precisely correct pathologic processes. Although the genomics at a single-cell level has been well documented to understand the cellular differentiation and lineage, it is still essential to study metabolome because it provides the most immediate and dynamic picture of the cellular functionality. Technically, mass spectrometry has promoted the development of single-cell metabolomics. However, there is a long-standing bottleneck that many unknown metabolites can’t be identified because their MS2 spectra couldn’t be acquired due to the pico-liter sample available in single cell analysis. In this manuscript, we designed a new ionization source to replace the commercial one. Over 1000 molecules and 600 MS2 spectra were acquired from single glioblastoma cells, and more than 300 phospholipids were successfully identified. With the rich information, single cancerous cells were readily distinguished from the normal cells. Significantly increased levels of unsaturated phospholipids were first observed in single cancerous cells. Moreover, our study first reports the differences of PC/PE isomers in cancerous and normal cells, especially at the single-cell level. This study can not only reveal new biomarkers, but also contribute to more life science studies that cell heterogeneities or cellular changes are concerned, such as stem cell differentiation and drug resistance of cancer cells.
Quantitative single-cell analysis enables the characterization of cellular systems with a level of detail that cannot be achieved with ensemble measurement. I am going to show some of our recent work on developing better approaches for single-cell sequencing, including whole genome amplification and whole transcriptome analysis. Whole-genome amplification (WGA) for next-generation sequencing has seen wide applications in biology and medicine when characterization of the genome of a single cell is required. High uniformity and fidelity of WGA is needed to accurately determine genomic variations, such as copy number variations (CNVs) and single-nucleotide variations (SNVs). Prevailing WGA methods have been limited by fluctuation of the amplification yield along the genome, as well as false-positive and -negative errors for SNV identification. I will present a new approach, emulsion WGA (eWGA), to overcome these problems. We divide single-cell genomic DNA into a large number (10^5) of picoliter aqueous droplets in oil. Containing only a few DNA fragments, each droplet is led to reach saturation of DNA amplification before demulsification such that the differences in amplification gain among the fragments are minimized. We demonstrate the proof-of-principle of eWGA with multiple displacement amplification (MDA), a popular WGA method. This easy-to-operate approach enables simultaneous detection of CNVs and SNVs in an individual human cell, exhibiting significantly improved amplification evenness and accuracy. Following similar design, we also develop a robust and simple single-cell RNA-seq method, named 'easier-seq' to perform whole transcriptome analysis. This new method is capable of capturing novel transcripts without polyA tails.

Keywords: Biomedical, Biotechnology, Genomics, Lab-on-a-Chip/Microfluidics
Application Code: Genomics, Proteomics and Other 'Omics
Methodology Code: Microfluidics/Lab-on-a-Chip
Biochemical Analysis at the Limits of Single Molecules and Single Cells
Building Massive Nanoelectrochemical Arrays for Imaging Neuronal Activity

In this talk, I will present our research on building and using massive nanoelectrochemical arrays to image dynamic release of neurotransmitter molecules from individual neuronal model cells. The key to this work is the development of fluorescence-enabled electrochemical microscopy or FEEM, a method which enables one to use fluorescence microscopy to monitor electrochemical current on massive electrochemical arrays. This method allows us to use arrays containing millions of nanoelectrodes to image dynamic redox processes such as generation and diffusion of redox molecules and collision and oxidation of individual metal nanoparticles on an electrode.

**Keywords:** Electrochemistry, Imaging, Nanotechnology, Neurochemistry

**Application Code:** Neurochemistry

**Methodology Code:** Electrochemistry
Mass spectrometry offers the most robust platform to discover and characterize new diagnostic, prognostic, and therapeutic biomarkers for ovarian cancer across all molecular classes. Moreover, a systems biology approach will allow the underlying biology of ovarian cancer to be understood. This presentation will discuss the challenges specific to the study of epithelial ovarian cancer (EOC) in humans and how these challenges have directed our thinking, in terms of the development of model organisms and mass spectrometry-based bioanalytical strategies. First, to augment the human model, we developed the domestic hen model of spontaneous EOC, which allowed us to longitudinally sample the rapid onset and progression of the disease in a controlled environment. Second, we developed bioanalytical tools to characterize structurally challenging analytes that are critical to a systems-level analysis. To increase the electrospray response of N-linked glycans, perform stable-isotope relative quantification, and semi-automated data analysis, we synthesized novel hydrophobic tagging reagents (INLIGHT™). This unique model organism has and continues to provide new insights into the biology of ovarian cancer; combined with other –OMICS data obtained through these novel bioanalytical approaches, we will understand the origin of ovarian cancer and ultimately translate that knowledge to humans.

Keywords: Biotechnology, Clinical Chemistry
Application Code: Clinical/Toxicology
Methodology Code: Mass Spectrometry
Ion mobility-mass spectrometry (IM-MS) has become a broadly applicable analytical method particularly in the study of protein structure through the determination of collision cross section (CCS). Although widely available, commercial IM-MS instruments suffer from low mobility resolution (defined as CCS/CCS) limiting their ability to analyze large proteins and protein complexes in the mobility dimension. Here, a homebuilt IMS, capable of mobility resolution ~65 in its alpha form and ~150 in its beta form, coupled to a commercially available Thermo Scientific Exactive Plus EMR Orbitrap MS is described. To further aid in protein structure determination, a variable temperature nano-ESI source was developed to study the thermal denaturation of ammonia channel (AmtB) in the presence of lipids.
In recent years, mass spectrometry has been applied to large molecular systems. Fragmentation of large protein and nucleoprotein complexes by solution disruption and by collision induced dissociation with gaseous targets provides some structural information but does not always provide full connectivity information and information on relative interface strengths within the complex. The Wysocki lab has developed the technique of surface induced dissociation, coupled with ion mobility dissociation and high resolution mass spectrometry as a tool to fragment protein and nucleoprotein complexes in a structurally diagnostic way. This talk will highlight progress made with a variety of instrument types (QTOF, Orbitrap, ICR). Challenges still faced in the development of SID in these instruments and possible solutions will be described. The use of complementary mass spectrometry approaches for overall structural characterization of macromolecular protein complexes will be highlighted.
Protein glycosylation is ubiquitous in biological systems and essential for cell survival. Aberrant protein glycosylation is directly related to human disease, including cancer and infectious diseases, and glycoproteins contain a wealth of information related to cellular developmental and disease statuses. However, due to the low abundance of many glycoproteins and heterogeneity of glycans, it is extraordinarily challenging to comprehensively analyze glycoproteins in complex biological samples. Based on the common features of glycans, we have developed chemical and enzymatic methods to globally analyze protein glycosylation by mass spectrometry (MS). Glycoproteins located on the cell surface are especially interesting because they frequently regulate extracellular events. In our lab, we specifically tagged surface glycoproteins for global and site-specific analysis. In combination with multiplexed proteomics, we quantified the dynamics of surface glycoproteins and measured their half-lives. Global analysis of protein glycosylation aids in a better understanding of glycoprotein functions and the identification of glycoproteins as disease biomarkers and drug targets.
Mass spectrometric imaging (MSI) provides a level of chemical and metabolomic information unmatched by any other imaging modality (including histopathology, MRI, and PET scans). Furthermore, MSI offers the potential for rapid and direct analysis of tissue even when an image is not of interest. This presentation will explore innovations in MSI and direct tissue analysis, focusing on sampling methods (including matrix-assisted laser desorption ionization MALDI and real-time in situ microextraction using the flowprobe) along with strategies for increasing the speed, spatial resolution, information content, and quantitative performance of the methods.

MSI takes advantage of the remarkable sensitivity and selectivity of mass spectrometry (including high resolution MS and tandem mass spec MS/MS and MS[^n^]). Furthermore, MSI can yield insight into hundreds of analytes in a single metabolomics analysis, without labeling. Even when a chemical “image” is not of interest, MSI techniques can provide rapid and direct analysis of tissue, including samples too small for classic GC/MS or LC/MS metabolomic analysis.

This lecture will explore the biomedical and biological applications of imaging mass spectrometry and direct tissue analysis, including the synergy between imaging MS and metabolomics, with examples from a wide variety of biomedical and metabolomic applications. These studies will include the potential for rapid screening for skin cancer (melanoma), assessment of liver allografts for liver transplantation, and investigation of treatment modalities in diseases such as Parkinson’s.

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

**Application Code:** Bioanalytical

**Methodology Code:** Mass Spectrometry
The specific recognition of ligands by proteins is a fundamental biological phenomenon, and the interaction between antigen and antibody in the immune system is a typical example. Recent advances in physical biochemistry have enabled us to describe what factors dominated the specificity and affinity of protein interactions. We have focused on several antigen-antibody interactions, including those specific for model antigens, e.g. hen lysozyme, toxin, and EGFR, and dissected the interactions from physicochemical viewpoints. Our conclusions could be summarized as follows. 1. Specificity is dominated by only a couple of residues, named hot-spot; 2. Hydrophobic interaction and/or a couple of hydrogen bonds are created by hot-spots, and cooperative binding of other paratope residues is induced via hot-spot interactions; 3. Other paratope residues are tolerant to site-specific mutation, and make incremental contributions to the interaction; 4. Variable domain interactions work as a cushion for fine-tuning of the paratope interface; 5. Interfacial water molecules make enthalpic contribution to the interaction, which complements the imperfect interfaces of antigen and antibody. 6. In principle, enthalpy change dominates the high affinity of antigen-antibody interaction, e.g. affinity maturation of the antibody. On the basis of these conclusions, we could propose one strategic scheme on improvement of antibody affinity for targets. These conclusions could be applied not only to improve the specificity and affinity of an antibody, but also to screen and/or design of small molecules, which can control specific biomolecular interactions. We would discuss the potential of physicochemical approaches as a ligand screening method.
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**Abstract Text**

In our previous work, we developed a multiplexed mass spectrometry method to quantify and localize proteins of interest in tissue sections and single cells. The method relied on monoclonal antibody tagging with unique rare-earth elements (REE-Ab). Using laser capture microdissection ICP-MS, we were able to detect and quantify proteins within a single cell.

Exciting advances in single nanoparticle analysis provided the necessary avenue for analyte quantification within a single cell. With this new capability, it is now possible to measure, with great accuracy, cellular uptake of exogenous metal ions, quantum dots, and REE-Ab tagged proteins. Using the high specificity of ICP-MS, new applications analyzing uptake of therapeutic metal-containing drugs, determine and assay for active metal ion homeostasis, and protein quantification. In this presentation, we will discuss current progress made using ICP-MS within life science applications and present data highlighting metal uptake into single cells and protein quantification.

**Keywords:** Atomic Spectroscopy, ICP-MS, Imaging, Immunoassay

**Application Code:** Bioanalytical

**Methodology Code:** Atomic Spectroscopy/Elemental Analysis
We introduce the new type viscometer EMS-1000 using the newly developed Electro-Magnetically Spinning (EMS) method. In the EMS method, a sample and a spherical probe made of aluminum are placed in a sample container, and the spherical probe in the sample is rotated using a rotating magnetic field generated outside the sample container to measure the rotation of the spherical probe in the sample. By observing the rotation of the spherical probe, you can investigate the rheological characteristics of the sample. Since the sample placed in the sample container does not come into direct contact with the device such as a detection mechanism, it has unique features such as "small sample volume", "non-contact manner and disposable", "sealed", "wide range" and which are not found in conventional viscometers.

In this report, we propose a new method of evaluating the properties of proteins with viscosity measurement using EMS-1000. It includes specific applications such as the correlation between protein concentration and its viscosity, the viscosity property of protein solution in several types of buffer solution, the observation of saccharification process with amylase and so on. It has become possible to continuously measure the change of viscosity that changes every moment even in a sample derived from biological samples such as protein solutions which were difficult to measure due to restrictions on the amount of sample or handling restrictions on the samples themselves.

We will introduce the potential of the EMS viscometer technology in the field of biochemistry which could not be applied to or was difficult to measure with conventional viscometer.

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**Keywords:** Biological Samples, Protein, Rheology

**Application Code:** Bioanalytical

**Methodology Code:** Physical Measurements
For monoclonal antibody development, scientists screen harvested cell culture samples for IgG titer analysis to identify the clones expressing most antibodies. Concentration of monoclonal antibody are estimated by Protein A affinity columns. A variety of monoclonal antibodies from harvested cell culture media can be captured and accurately quantitated by Protein A affinity chromatography. If necessary, a partial purification can be accomplished using an affinity protein A column for further analysis. We have used a 20 \( \mu \text{m}, 4.6 \text{ mm ID} \times 3.5 \text{ cm} \) column for high performance affinity chromatography for our study. Earlier we reported wide dynamic range with high sample concentration load for mAb quantitation, fast analysis of monoclonal human IgG1 within 2 min, high sensitivity for mAb titer determination and long lifetime of more than 2000 injections per column with no signs of deterioration (Ref: WCBP 2017 Poster). In this presentation we report the rapid purification of a varieties of IgG subclasses such as IgG1, IgG2, IgG4 as well as IgGs from a number of different sources such as human, mouse, rat and rabbit. Analysis could be completed within 2 minutes. Because the recombinant protein A ligand used in this column is a code-modified hexamer of the C domain, this column has an affinity for various antibodies that the native protein A and some other recombinant protein A ligands do not possess. The IgG peak fraction was collected and subjected to size exclusion chromatography for further testing of its purity and aggregate analysis. The result of the analysis indicated that only IgG was present in this fraction (data not shown). Rapid separation and robust quantification of antibodies could be carried out using this Protein A affinity chromatography column.

**Keywords:** Bioanalytical, HPLC Columns, Liquid Chromatography, Protein

**Application Code:** Bioanalytical

**Methodology Code:** Liquid Chromatography
Liquid chromatography – tandem mass spectrometry (LC-MS/MS) has been a staple for protein quantitation, but generally through the use of bottom-up methods. Top-down methods have generally been reserved for discovery research. Recently, we demonstrated the potential for using LC-MS/MS on a triple quadrupole mass spectrometer (QQQ-MS) in multiple reaction monitoring (MRM) mode for intact top-down protein quantitation (J. Amer. Soc. Mass Spectrom. 2016, 27, 886-896). This approach could hold new promise for streamlining protein quantitation by avoiding proteolytic digestion steps, but there are some hurdles that need to be addressed. Our work has shown that protein ion transmission in the QQQ-MS is a bit different than what is known for small molecules (J. Amer. Soc. Mass Spectrom. 2017, 28, 1977-1986). Also, less is known about maximizing electrospray ionization efficiency for intact protein quantitation. Additionally, the QQQ-MS typically has a limited mass range and it is unclear how large of a protein can be accommodated for analysis. From the stand-point of reversed phase separations, there are limited means by which the selectivity of separations can be altered. Recently, we have begun to explore these latter aspects. Reported will be our current efforts for increasing the applicability of LC-MS/MS and QQQ-MS for top-down protein quantitation. This includes a survey of different additives that can affect protein separation and ionization.

Keywords: Bioanalytical, Liquid Chromatography/Mass Spectroscopy, Protein, Quadrupole MS
Application Code: Bioanalytical
Methodology Code: Liquid Chromatography/Mass Spectrometry
We summarize recent progress with our Continuity™ mass spectrometers. Our miniature series mass spectrometer was revised to offer improved performance and reliability. Enhanced interface and control features were added to the design to allow integration with external systems, such as laser-sampling systems, gas- or liquid chromatographs, etc. Compound testing was also expanded from pesticides and illicit drugs to now include chemical warfare agents (CWA). Many technical improvements were made to our external sampling system, the thermal desorption - electrospray ionization (TD-ESI) source. The TD-ESI now features greater reliability and increased high-voltage output. The TD-ESI system was modified to accommodate additional ionization techniques such as corona discharge and atmospheric pressure chemical ionization (APCI). Significant improvements were made with our Continuity mass spectrometers that include greater vacuum control, data-dependent acquisition, and enhanced ruggedization for field deployment. Compound testing was also expanded for CWA and peptide samples to better demonstrate the broad range of applications supported by the Continuity platform.

Keywords: Mass Spectrometry
Application Code: High-Throughput Chemical Analysis
Methodology Code: Mass Spectrometry
Miniaturization of mass spectrometers, and sector instruments in particular, leads to a trade-off between throughput and resolution resulting in poor performance relative to laboratory instruments, limiting their use in the field. The use of spatially coded apertures in sector mass spectrometers can eliminate this trade-off. Spatial aperture coding replaces the single slit in a traditional sector spectrometer with a one- or two-dimensional array of slits. The size of the smallest slit in the array sets the resolving power, while the total open area determines the throughput. Traditional spectra can then be computationally reconstructed from coded spectra given knowledge of the aperture pattern and physics of the mass analyzer. The enhanced throughput can increase the signal level with respect to the underlying noise, thereby significantly improving sensitivity to low concentrations of analyte. As resolution is maintained, there is no decrease in selectivity. This paper discusses the state of development of spatial aperture coding in mass spectrometry. Both one- and two-dimensional codes have been demonstrated in a simple 90-degree magnetic sector mass analyzer, demonstrating up to an order of magnitude signal increase without loss of resolution. In addition, recent work adapting both Mattauch-Herzog and cycloidal mass analyzers for aperture coding are described. Results are presented from a portable cycloidal coded aperture miniature mass spectrometer (C-CAMMS) prototype incorporating aperture coding, a new magnet geometry with improved field uniformity, a miniature carbon nanotube (CNT) field emission electron ionization source, and a capacitive transimpedance amplifier ion array detector, showing a >10x increase in throughput with no loss in resolution.
We present a summary of mass spectrometers recently developed at NASA for planetary exploration. Purpose-built instruments must survive the rigors of space while using minimal weight, power, data rates, and human intervention. Current and future missions to bodies such as Mars and the Ocean Worlds of Enceladus, Europa, and Titan, among others, seek the molecular and isotopic signs of habitability and potentially life itself in unknown solid, liquid, or gaseous samples. As such these mass spectrometers must be designed for broad sensitivity to potential analytes at low concentrations in complex matrices. The Mars Organic Molecule Analyzer (MOMA) ion trap mass spectrometer in development for the ExoMars rover approaches this challenge through a combination of front-end designs (gas chromatography, laser desorption) and molecular isolation and structural characterization protocols.
Atmospheric pressure (AP) ionization and gas chromatography (GC) mass spectrometry (MS) are two major techniques widely used in practical applications of environmental mass spectrometry. The former approach is presented by a portable MT Explorer 50 (MTE50) ion trap mass spectrometer. This presentation describes features of MTE50 which are important for its miniaturization including atmospheric pressure interface with direct introduction of atmospheric ions into a multipole ion guide and the use of hydrogen as a buffer gas. In our newest development a miniaturized version of MTE50 is coupled with a MEMS-based GC to accommodate analysis of complex mixtures of volatile and low-volatile chemical compounds. This new miniature GC-MS instrument features a full mass range of 35-450 Da at about 1 L volume. Its design features and performance characteristics will be discussed.

Keywords: GC-MS, Instrumentation, Mass Spectrometry, Portable Instruments
Application Code: Environmental
Methodology Code: Mass Spectrometry
Mini Mass Spectrometers

Miniature Mass Spectrometer with Triple-Quad Capabilities for Structural and Quantitative Analysis

Miniature mass spectrometers have been developed for in-field, fast analysis. The use of the ambient ionization method enabled direct analysis of complex biological samples using miniature MS systems without traditional complicated lab-procedures. The atmospheric pressure interfaces supported by compact pumping systems are also critical for combining the ambient ionization sources with the portable systems. Recently, research effort has been put for developing the capability of the miniature MS systems to perform various tandem MS analysis modes, which are routinely applied with commercial triple quadrupole mass spectrometer. In this presentation, the MS/MS scan modes implemented with a newly developed miniature MS system will be presented, including multi-reaction monitoring, precursor scan, neutral loss scan, etc, and their application for qualitative and quantitative analysis. The associated instrumentation development will also be reported.

**Keywords:** Bioanalytical, Biomedical, Instrumentation, Mass Spectrometry

**Application Code:** Biomedical

**Methodology Code:** Mass Spectrometry
Activity-based sensing (ABS) is an emerging field that utilizes chemical reactivity, rather than conventional lock-and-key molecular recognition, to probe and manipulate biological systems. We are advancing the ABS concept and applying it to discover and decipher the biology of new chemical signals, including reactive oxygen, sulfur, and carbon species and transition metals. This presentation will describe our latest results on a new paradigm of transition metal signaling, where essential elements like copper and iron can serve as dynamic regulators of biological function in addition to their traditional roles as static metabolic cofactors.

Keywords: Biosensors, Fluorescence, Metals, Neurochemistry
Application Code: Biomedical
Methodology Code: Fluorescence/Luminescence
Molecules and Materials for Advanced Bioimaging and Diagnostics

Inorganic Complexes for $^{19}$F Magnetic Resonance Bioimaging

$^{19}$F Magnetic resonance imaging (MRI) is an emerging technique for in vivo imaging, enabling ‘hot spot’ imaging of environments of interest due to the absence of endogenous fluorine signals in biological specimens. Diamagnetic species such as perfluorinated carbons have been used extensively for this imaging modality, and can be used for a range of applications including imaging of lungs and O$_2$ tension. A limitation of this technique is the lengthy acquisition time required due to low fluorine concentrations and long T$_1$ relaxation times. We are developing a number of imaging agents based on paramagnetic metal complexes ($\text{Cu}^{2+}$, $\text{Co}^{2+}$, $\text{Ni}^{2+}$, $\text{Fe}^{3+}$, $\text{Gd}^{3+}$) that shorten the relaxation times of nearby fluorines. These scaffolds are being explored for a number of applications including agents for rapid imaging acquisition of biological targets, multicolor imaging, and environmental sensing.

Keywords: Biomedical, Biosensors, Magnetic Resonance
Application Code: Biomedical
Methodology Code: Magnetic Resonance
Molecules and Materials for Advanced Bioimaging and Diagnostics

From Light to Sound: Photoacoustic Probes for Non-Invasive In Vivo Imaging

To produce soundwaves in tissue, which can be detected and converted into high-resolution 3D images. Since sound scattering is low in tissue, this technology has the potential to resolve approximately 1/200 of the imaging depth; in other words, single mammalian cells of 20 μm diameter can be detected at a depth of 4 cm using PA imaging. By comparison, optical based methods such as fluorescence imaging is typically constrained to imaging depths in the mm range. Figure 1a and 1b depict the flank of a live mouse imaged with fluorescence and photoacoustic tomography, respectively, demonstrating the superior imaging resolution possible with this technology. Although this approach can uncover important details in vivo, there is a dearth of stimuli-responsive probes for this imaging modality. In this seminar, we will first discuss the strategies employed to construct PA probes, as well as design criteria and highlight examples from our laboratory which have been used to visualize important biological processes in vivo.

Keywords: Biomedical, Fluorescence, Imaging, Photoacoustic

Application Code: Biomedical

Methodology Code: Sensors
Current key questions in medical research require an understanding of how the chemistry of biological systems changes in health and disease. This can be achieved by the preparation of molecular imaging tools, which can report on specific aspects of their chemical environment. Our research is focused on the development of magnetic resonance (MR) and fluorescent sensors that can report on redox state or metal ions in biology. In particular, we seek to develop sensors that are reversible, selective, and provide unambiguous outputs.

Reversible fluorescent sensors enable the imaging of biological systems over time. We have developed a family of probes based on flavins that reversibly sense oxidation and reduction, and have applied them to study various biological questions. Amongst these probes is FCR1, a ratiometric sensor, which operates by a Förster resonance energy transfer (FRET) mechanism, and undergoes a reversible change from blue to green upon oxidation.

Selectivity of fluorescent sensors is essential in studying an individual species in a complex chemical environment. Sensors must not only be selective for the species of interest, but their response must be unaffected by the presence of any other species. Selectivity is therefore an important consideration in studying the metal ions in biology. We have developed sensors that exhibit selectivity for monofunctional platinum complexes over all other platinum species, and have prepared tools for the bioavailable copper pool.

One of the challenges in responsive magnetic resonance imaging (MRI) is the residual background signal from gadolinium MR contrast agents, even in “off” form. We have developed cobalt complexes that can unambiguously report on changes in oxygen environment or anion concentration by MRI.

Keywords: Biomedical, Fluorescence, Imaging, Magnetic Resonance

Application Code: Biomedical

Methodology Code: Fluorescence/Luminescence
Herein, we describe phase change materials capable of accumulating within tumor tissue. The materials are Pt-drug loaded enzyme-responsive nanomaterials containing isotopic labels and near-infrared (NIR) fluorophores, enabling detailed tracking of the materials within cells, and within tissues. In this work, we demonstrate the use of optical and isotopic techniques to independently determine, with nanometer resolution, the localization of both drug and carrier in diseased tissue from a living organism. Amphiphilic block copolymers were generated from hydrophobic Pt(II) prodrugs and 15N-labeled inert moieties, together with hydrophilic peptide substrates for matrix metalloproteinases (MMPs) and NIR dyes. Dialysis of these copolymers yielded nanoparticles with peptide shells and drug-loaded cores (Pep-Pt-NPs) that undergo a nanometer to micrometer scale morphology change upon exposure to MMPs in tumor tissue. Fluorescence microscopy analysis was performed on live animals in vivo, on whole organs ex vivo, and on tissue sections to characterize the macroscopic, microscopic, and nanoscopic distribution of the fluorescently labeled nanocarrier. To independently evaluate the distribution of drug and carrier in tumor tissue, the localization of the isotopically labeled polymer backbone was compared to that of Pt by nanoscale secondary ion mass spectrometry (NanoSIMS). The correlation of this nanoscaled isotopic technic with super-resolution fluorescence microscopy revealed the release of the drug from the nanocarrier and co-localization with its proposed target—the cellular DNA within tumor tissue. This study demonstrates the potential of correlative techniques for studying interactions of drugs and nanoparticles within relevant, complex biological structures. Such studies are essential for designing nanomaterials and understanding their performance following in vivo delivery.

Keywords: Biomedical, Biotechnology, Fluorescence, Materials Characterization
Application Code: Biomedical
Methodology Code: Microscopy
Over two decades ago, nucleic acid therapeutics emerged as a promising new technology for treating diseases with a known genetic basis. In addition, in recent years, nucleic acids have been identified as sequence-specific regulators of the immune system. However, issues pertaining to their stability, toxicity, and delivery have dramatically limited their application and thus slowed the growth of the field. Indeed, the vast majority of nucleic acid drugs that are currently being developed target diseases that can be addressed in the liver, the site where nucleic acids accumulate when systemically administered. We have discovered a new form of nucleic acids that can be actively internalized by most cell and tissue types, without the need for transfection agents, owing to their novel nanoscale architecture. These new forms of DNA and RNA, referred to as spherical nucleic acids (SNAs), are revolutionizing the way we study, track, and treat disease. SNAs are being used as potent gene regulation agents that can be topically delivered to the skin and cross the blood-brain-barrier to treat neurodegenerative conditions; they have also shown immense promise as new cancer vaccines. This presentation will describe how SNAs are steering the community toward a whole new way of thinking about digital drug design.
The exact biomolecular makeup of our body may be the most important aspect of biology and Health. Traditionally our information has been based on ex-vivo samples, from blood tests to biopsies, followed by biochemical analysis and/or microscopic imaging. These approaches lose much of the important information, i.e., precise 3-dimensional mapping as a function of time. In principle, only 4-dimensional (4d) space-time imaging could obtain such a complete biomedical/molecular picture. We introduce here a new 5-dimensional imaging mode, where the 5th dimension is the biochemical/molecular identity. Like other imaging methods, it uses contrast agents, specifically targeted nano-agents or nano-probes.

Relating to cancer therapy, tumors are usually biochemically anomalous due to (1) acidosis (low pH), (2) hypoxia (low oxygen) and/or (3) hyperkalemia (high extracellular potassium). The first makes them resistant to many forms of chemotherapy; the second suppresses any form of radiation therapy (from proton beam to photodynamic) while the third has only recently been shown to suppress immunotherapy. Thus such chemical/molecular information, including its 3-dimensional map at a given point in time, may be extremely useful to the caretaker who has to decide on the proper mode of therapy, i.e. therapeutic prognosis (Theragnosis), as well as monitor its success, when involved in personalized (precision) medicine. Notably, the same nanoprobes could be used for nano-theragnostics.

We report here on our ongoing work regarding such 4d molecular tumor photoacoustic imaging (PAI) of pH, O2 and K+. This includes animal tests, as well as comparisons with optical absorbance and fluorescence imaging, all done with the aid of specially designed nanoprobes, e.g. ion-selective photoacoustic nano-optodes, as well as novel PAI methods, all tailor made for the molecular imaging of such bio-analytes, with the aim of successful in-vivo molecular imaging and cancer nano-theragnosis.

Keywords: Imaging, Nanotechnology, Photoacoustic
Application Code: Nanotechnology
Methodology Code: Microscopy
We introduce biomolecular payloads into cells for gene editing at high throughput for off-the-shelf solutions targeting hemoglobinopathies, immune diseases, and cancers. We circumvent the need for viral transfection and electroporation, both of which have significant disadvantages in safety, throughput, cell viability, and cost. Mechanical deformation can make cell membranes transiently porous and enable gene-editing payloads to enter cells. These methods use specific chemical functionalization and control of surface contact and adhesion in microfluidic channels. Likewise, penetration of reproducibly nanomanufactured, loaded sharp features can introduce these packages into individual or many cells. We discuss our progress with these approaches and the methods that we use to quantify success.
AIEgens as Weapons for Fighting against Diseases

Recently, a new class of fluorogens with aggregation-induced emission characteristics (AIEgens) has attracted great interest in biological and biomedical applications. Different from traditional fluorogens which show an aggregation-caused quenching (ACQ) phenomenon at high concentrations, AIEgens observed an opposite phenomenon and identified the restriction of intramolecular rotation (RIR) as the main reason for the AIE effect. Guided by the RIR mechanism, we have developed a series of new AIE materials with emission colors covering the whole visible spectrum, fluorescence quantum yields up to unity. Owing to their high emission efficiency, large absorptivity, good biocompatibility, excellent photostability and superb selectivity, the AIEgens have been applied to long-term non-invasive in vitro and in vivo cell tracing, cell apoptosis detection, chemotherapy, drug delivery, and bacterial imaging (detection, screening). Some AIEgens showed efficient reactive oxygen species (ROS) generation in the aggregates, which offer the unique opportunity to develop light-up probes for image-guided photodynamic inactivation (PDI) of bacteria and photodynamic cancer therapy. AIEgens have many advantages in bioimaging, diagnosis and therapy applications.

Keywords: Biomedical, Biosensors, Fluorescence, Material Science
Application Code: Biomedical
Methodology Code: Fluorescence/Luminescence
Nanoscale Assembly of Nucleic Acids and Peptides for Live Cell and In Vivo Molecular Imaging

Approaches for intracellular and targeted delivery of nucleic acids and peptides for detection and regulation of gene and protein expressions are essential for cell biology and medicine. We have developed several types of nanoscale assemblies of DNA and peptides, which enable efficient delivery of DNA or peptide probes into tumor cells. Based on these assemblies, signal amplification reactions such as hybridization chain reaction (HCR) and catalytic hairpin assembly can be realized in living tumor cells, allowing ultrasensitive detection of varying molecular targets in living cells and in vivo.

Genetic encoded RNA sensors can be engineered and stably expressed in living tumor cells within a designed tRNA scaffold using a light-up RNA aptamer binding to sulforhodamine and separating the dye from a contact quencher. Co-expression of the RNA sensor with GFP allows dual-emission, ratiometric imaging of target microRNA in living cells. Moreover, Stimulus-responsive, self-assembling peptide nanoparticles has been constructed using an identified short ELP peptide, affording a new biocompatible and sensitive agent for peptidase imaging and theranostics. Our results indicate that the developed nanoassemblies may provide useful platforms for developing molecular imaging agents and theranostics tools against cancer.

Keywords: Biosensors, Imaging, Nucleic Acids, Peptides
Application Code: Bioanalytical
Methodology Code: Sensors
The National Institute of Justice (NIJ) is the research, development and evaluation agency of the U.S. Department of Justice. NIJ’s Office of Investigative and Forensic Sciences maintains a program of external funding for R&D in forensic sciences. This program is a leading federal funder in this mission space, and the portfolio spans a broad range, from fundamental research, to development of prototype devices, to validation of novel instruments and methods.

Forensic science is a collection of applied disciplines that draws from all branches of science. Nevertheless, forensic scientists most often tend to be concerned with the detection, collection, separation, and analysis of biological and chemical samples. Because of the unique circumstances of forensic evidence, there is an ongoing need for these analyses to be done on ever smaller, degraded or mixed samples. Balancing that is the need to ensure that analytical methods applied to these challenging samples are objective, rigorously tested, and foundationally valid. These needs drive NIJ’s continuing R&D investments in analytical chemistry and bioanalytical science. Advances in mass spectrometry, electrophoresis, applied spectroscopies, microscopy and microfluidics, among other analytical techniques, have yielded or show promise for successful application to forensics. NIJ anticipates continued interest in advancing these technologies, as well as emerging analytical methods, for forensic application. In this effort, NIJ strives to engage the analytical chemistry and applied spectroscopy research communities to bring novel perspectives to solving forensic problems.

An overview of NIJ’s research and development portfolio will be presented, highlighting funding opportunities, including the anticipated plans for the FY2018 Research and Development in Forensic Science for Criminal Justice Purposes solicitation and examples of past funded projects in areas relevant to the analysis of trace evidence materials.

Keywords: Forensics
Application Code: Homeland Security/Forensics
Methodology Code: New Method
You are an investigator that has recovered an expensive artifact to the rightful owner or you are faced with a crime scene and want to retrace what has transpired, how to do this? Basically one collects as many pieces of evidence as possible and then reconstruct the scene or rightful owner of the object based on these clues. Objects or rooms contain many clues about how they are used and the lifestyle characteristics (e.g. food preferences, personal care preferences, medications, etc) of the people that use them. The vast majority of these clues (I am fairly sure >99.99%) remain hidden and are therefore not used by investigators. In this presentation we will highlight how mass spectrometry, combined with molecular networking, creating reference data sets that reflect lifestyle characteristics and 3D cartography can begin to reveal such lifestyles but also how such lifestyle characteristics can be used to “reconstruct” the typical use within a building - a step toward crime scene reconstruction from chemical clues. Finally we will discuss why they are not routinely used and what it will take to get these capabilities into real life applications from a community and chemoinformatic and computational standpoint.

Keywords: Mass Spectrometry, Trace Analysis, Metabolomics, Metabonomics
Application Code: Other
Methodology Code: Mass Spectrometry
The composition of microbial organisms associated with skin is unique to an individual. This is because the experiences each person has since birth are unique, and it is these physical interactions with the world that allow microbes to colonize and form communities ('microbiomes') on our bodies. Even identical twins, whose microbiota are significantly more similar than other siblings, each have a unique profile.

While, an individual’s core microbiome is considered stable by the age of 2-3, it can still undergo variation as we change aspects of our lifestyle that cause us to be exposed to different microbial worlds. The skin microbiome is our primary interface with the world and the interface we most readily leave behind when we interact with a space.

To date, the evidence to support this has been limited by small studies and anecdotal enquiry. We are performing a systematic analysis of a human population around Miami, FL, to determine categorically whether elements of their lives can be predicted from their microbiome, both on their bodies, and that left behind on surfaces they interact with. In doing so, we will create a list of highly specific microbial biomarkers for particular traits (e.g. young adult female vegetarian, who lives in the suburbs and works in a bakery or bread counter). We will also build a sophisticated artificial neural network and database to enable extrapolation of microbial signature detection to other samples, so that a person’s traits can be detected from the microbial community they leave behind. This proof of principle study will form the foundation of a forensic effort in Miami to create a new suite of trace evidence options that can be leveraged by investigators to help shape their interpretation of a crime scene.

Keywords: Forensics, Neural Network, Nucleic Acids
Application Code: Homeland Security/Forensics
Methodology Code: Data Analysis and Manipulation
The discipline of toolmark analysis is based on the observed phenomenon that through force and contact individual tools can transfer tool-specific marks to a target object. In the case of firearm forensics it is the firearm which leaves unique marks on cartridge cases and bullets. Microscopic examination of these marks allows firearm examiners to assess the likelihood of common origin (e.g. linking a cartridge case found at a crime scene to a test fire from a suspect’s firearm).

Several 3D scanning technologies, including our recently developed TopMatch scanner, are capable of measuring a high-resolution 3D surface topography in standard units. These topographies represent a one-to-one geometric mapping between the scanned digital surface and the actual physical surface. Virtual Microscopy, or the examination of digital representations of objects rather than the physical objects themselves, offers many novel use cases to the forensic examiner. These include new abilities in remote data sharing/collaboration, visualization, annotations, verification, and proficiency testing.

In support of Virtual Microscopy, we developed specialized viewing software, ran a training workshop at a national meeting, and conducted a blind validation study. Each participant utilized the same computer setup loaded with a training tutorial and two virtual CTS-like proficiency tests. Unlike a traditional physical proficiency test, Virtual Microscopy has no inter-operator test set variability. Participants were asked not only to complete a standard results worksheet but also to use the software to indicate regions of toolmark similarity via a ‘paintbrush-like’ feature in the software.

In this presentation, we will share both details of our work in Virtual Microscopy as well as study results. Our work demonstrates the feasibility of the method and represents an important step towards validating this new technology for use in casework and proficiency testing.

Keywords: Forensics, Identification, Microscopy, Software
Application Code: Homeland Security/Forensics
Methodology Code: Surface Analysis/Imaging
Vibrational spectroscopy and Advanced Statistics for Detection and Characterization of Gunshot Residue

Vibrational spectroscopy including Raman microscopy and FTIR has numerous applications in modern forensic chemistry. Vibrational spectroscopic characterization allows for confirmatory class identification of analytes through its high specificity towards molecular structure and composition. The technique is non-destructive, rapid, sensitive and requires little or no sample preparation. Furthermore, portable Raman and FTIR spectrometers are readily available, allowing for crime scene accessibility. Vibrational spectroscopy offers several advantages over the current methodology for GSR analysis. The technique has been shown to detect components from both the organic and inorganic constituents of GSR on adhesive tape. This is contrary to current GSR elemental analysis methods which rely solely on the detection of the heavy metals (lead, barium and antimony). This is problematic since environmental concerns have led to the increased popularity in heavy metal free or “green” ammunition.

The firearm discharge process could be considered analogous to a complex chemical reaction. Therefore, the chemical composition of the products (GSR particles) is directly related to the chemical nature of the reagents (firearm-ammunition combination) and the conditions of the reaction. Preliminary results show that Raman and FTIR data collected from GSR particles originating from different firearm-ammunition discharges were successfully classified according to caliber.

This project was supported by Award No. 2016-DN-BX-0166 awarded by the National Institute of Justice, Office of Justice Programs, U.S. Department of Justice. The opinions, findings, and conclusions or recommendations expressed in this publication are those of the authors and do not necessarily reflect those of the Department of Justice.

Keywords: Forensics, Raman Spectroscopy, Statistical Data Analysis, Vibrational Spectroscopy
Application Code: Homeland Security/Forensics
Methodology Code: Vibrational Spectroscopy
Recent Advances in Laser Induced Breakdown Spectroscopy

Development of a LIBS Database for the Forensic Interpretation of Glass Evidence

The aims of this project are to standardize the analysis and interpretation of glass evidence using Laser Induced Breakdown Spectroscopy (LIBS). A novel quantitative analysis strategy incorporating the integration of the transient LIBS signal is also presented. The quantitative results are compared to results from a parallel study involving the analysis of 420 vehicle glass samples using Laser Ablation-Inductively Coupled Plasma-Mass Spectrometry (LA-ICP-MS). A likelihood ratio approach to evidence interpretation, through the use of a LA-ICP-MS glass database, has been shown to yield low error rates for the assignment of weight of glass evidence. However, LA-ICP-MS is a costly, complex analytical technique and may be impractical to implement in every forensic laboratory for routine casework. This presentation demonstrates that LIBS may provide an inexpensive alternative that yields comparable discrimination power to LA-ICP-MS for the elemental analysis of vehicle glass comparisons.

The likelihood ratio provides a continuous numerical, quantitative and objective, assessment of evidence interpretation. However, the estimation of a likelihood ratio requires a database. At Florida International University (FIU), a large vehicle glass collection of up to 538 samples is available in order to generate a LIBS glass database to be used for likelihood ratio estimates.

The results presented include a) the development of a novel methodology for the quantitative analysis of glass using LIBS, b) compare the analytical figures of merit between LIBS and LA-ICP-MS, c) describe the LIBS glass database of the 420 samples, d) calculations of likelihood ratios based on the database, and e) propose an overall strategy for the analysis and interpretation of glass evidence using LIBS that can be defended in court. This work is funded under the NSF IUCRC Center for Advanced Research in Forensic Science (CARFS).

Keywords: Forensic Chemistry
Application Code: Homeland Security/Forensics
Methodology Code: Atomic Spectroscopy/Elemental Analysis
The use of laser-induced breakdown spectroscopy (LIBS) in biomedical and biological applications has increased dramatically in the past decade. It is the potential to make rapid in vivo or in situ measurements that is driving the interest in medical or biomedical applications, while the ability to make localized point elemental composition measurements on small fragile biological specimens with minimal or no sample preparation is a primary factor in the technique’s application to bioanalysis.

However, the nature of biological specimens including a high degree of heterogeneity and a complex matrix structure introduces complexities that might not exist during the LIBS analysis of more uniform substrates (e.g. alloys, homogenous fluid media, or minerals). As a consequence, the more typical performance observed for LIBS measurement may be degraded. Such complexities will be illustrated using our experience in the biological systems of bacterial cells; human fingernails; and fish otoliths. The problems that arise during testing in such systems and solutions to these obstacles will be described.

Keywords: Atomic Spectroscopy, Biological Samples, Biomedical, Laser
Application Code: Bioanalytical
Methodology Code: Atomic Spectroscopy/Elemental Analysis
Recent advances in laser induced breakdown spectroscopy

Improvement of LIBS Quantitative Capability Using Collinear Long and Short DP Laser

Recent years have seen strong demands for better efficiency in several types of commercial plants including steel-making processes, boilers, gas turbines and so on. The understanding of the controlling factors is becoming more important to improve the efficiency of industrial systems. The so-called “standard methods” are well established and easily accessible, however they are limited in terms of meeting the industrial needs described above because of slow response, low sensitivity, complicated pre-concentration, etc. In contrast, laser diagnostics makes it possible to monitor these parameters due to their fast response, high sensitivity, and non-contact features. For example, laser-induced breakdown spectroscopy (LIBS) has been used in various applications to meet the practical industrial requirements noted above.

In this study, a new collinear long and short DP-LIBS method was proposed to improve the detection ability and the measurement accuracy by the control of the plasma cooling process using the long pulse-width laser radiation. Fig.1 shows the notional comparison of laser-induced plasma processes of single-pulse LIBS (SP-LIBS) and long and short DP-LIBS. The plasma generated by the short pulse-width laser is stabilized and maintained at high temperature during the plasma cooling process by long pulse-width laser radiation. The combination of LIBS with a 3D profile detection system was also proposed to maintain the quantitative capability of LIBS for the industrial processes, in which target profiles are associated with change in shape and distance. The combination of LIBS with a 3D profile detection system is important because the focus of a laser beam is one of the most important factors of LIBS. The system design of LIBS with a 3D profile detection apparatus enables the automated adjustment of the laser beam focus point and has a potential of practical LIBS applications to the industrial processes such as iron-making plants.

Keywords: Atomic Emission Spectroscopy, Laser, Monitoring, Process Control
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Atomic Spectroscopy/Elemental Analysis
Monitoring the contents of subsurface fluids poses numerous challenges to the sensor community. For example, the subsurface environment is notoriously harsh, with large potential mechanical, thermal, and chemical stresses, making long-term stability and survival a challenge to any potential in situ monitoring method. Laser induced breakdown spectroscopy (LIBS) has been demonstrated as a promising technology for chemical monitoring of harsh environments and hard to reach places. LIBS has a real-time monitoring capability and can be used for the elemental and isotopic analysis of solid, liquid, and gas samples. The flexibility of the probe design and the use of fiber-optics has made LIBS particularly suited for remote measurements. The paper focuses on developing a LIBS instrument for downhole high-pressure, high-temperature brine experiments, where CO2 leakage could result in changes in the trace mineral composition of an aquifer. The progress in fabricating a compact, robust, and simple LIBS sensor for widespread subsurface leak detection is presented.

Keywords: Atomic Emission Spectroscopy, Environmental Analysis, Environmental/Water, Laser
Application Code: Environmental
Methodology Code: Atomic Spectroscopy/Elemental Analysis
This presentation will describe the experimental conditions associated with the slurry measurement to achieve good precision of the laser induced breakdown spectroscopy (LIBS) measurement. The goal of this project is to assist the Defense Waste Processing Facility (DWPF) at the Savannah River Site (SRS) in accelerating DWPF melter operations. The capability of direct analysis of slurry will significantly increase analytical throughput and will reduce waste generation in radiological analytical facilities, providing analyses suitable for waste acceptance and production records. Laser Induced Breakdown Spectroscopy (LIBS) will serve as the basis for the system for direct analysis of DWPF Sludge Receipt and Adjustment Tank (SRAT) product (slurry consisting of only sludge or waste). The main issues to be resolved with LIBS analysis of liquid samples are poor detection sensitivity and precision. Because water can quench the laser plasma and suppresses the LIBS signal, poor sensitivity may result. Large standard deviations for LIBS liquid data are due to the laser induced shock wave caused by turbulence on the liquid surface. Slurry samples contain a large amount of water and large particle sizes. The effects of water content and particle sizes on LIBS measurement will need to be studied. To improve LIBS' reproducibility and detection limits for slurry measurements, various experimental parameters which can affect LIBS' analytical figure of merit were studied. The study shows that by using the appropriate slurry sample handling systems, optimum experimental parameters and some data processing techniques, reasonable accuracy and precision can be achieved. For minor elements, Current work demonstrates that LIBS has the potential to be developed for on-line analysis and control of DOE wastes slurry processing. However, further work on improving signal sensitivity and data reproducibility is needed.

Keywords: Atomic Emission Spectroscopy, Atomic Spectroscopy, Elemental Analysis, Environmental
Application Code: Environmental
Methodology Code: Atomic Spectroscopy/Elemental Analysis
Spatially Resolved Molecular Analyses of Biological Systems

Ambient Imaging of Biological Samples Using Nanospray Desorption Electrospray Ionization (nano-DESI) Mass Spectrometry

We have developed nanospray desorption electrospray ionization (nano-DESI), an ambient ionization technique that relies on localized liquid extraction of analyte molecules from the sample followed by soft ionization of extracted species at a mass spectrometer inlet. Nano-DESI enables quantitative mass spectrometry imaging (MSI) of biomolecules in fully hydrated samples with minimal or no sample pre-treatment. Furthermore, simultaneous imaging of sample topography and chemical composition has been achieved by coupling nano-DESI MSI with shear force microscopy. This multimodal imaging approach has enabled imaging of living microbial communities with complex topography and high-spatial-resolution imaging of tissue sections. Coupling of nano-DESI MSI with other imaging modalities including scanning ion conductance, optical, and fluorescence microscopy is currently underway in our laboratories. Collectively, this multimodal imaging approach will provide unprecedented depth of chemical information produced in each imaging experiment. Specifically, chemical gradients obtained using nano-DESI MSI will be mapped onto sample topography, spatial localization of selected redox-active species including reactive oxygen species (ROS) and redox-active metabolites and spatial localization of natural chromophores and fluorophores in complex biological systems. The design principles and performance of the new imaging platform will be presented and multimodal imaging of tissue sections and other biological samples will be demonstrated.

Keywords: Imaging, Instrumentation, Mass Spectrometry, Method Development

Application Code: Bioanalytical

Methodology Code: Mass Spectrometry
This presentation will describe our effort to achieve high-throughput genomic analysis of single cells in a complex tissue. Our approach is enabled by a new family of fluorescent probe, semiconducting polymer dots, with which we use to barcode single cells or to visualize the transcripts from single cells. We will describe our current progress and discuss potential applications and future developments.

**Keywords:** Bioanalytical, Biomedical

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

**Methodology Code:** Microscopy
Mass spectrometric imaging is a powerful tool enabling one to plot a molecular distribution across a sample. Sample preparation however is difficult and tedious, but yet critical to data/image quality. We are examining the use of superhydrophobic materials that are patterned with hydrophilic patches to aid in sample preparation for mass spectrometry. Superhydrophobicity arises from a hierarchical surface structure with features on both the micrometer and nanometer scales. Recently, a number of novel commercial superhydrophobic coatings based on functionalized silica nanoparticles have become available for a range of consumer applications. Superhydrophobic surfaces, characterized by water contact angles in excess of 150 degrees and sliding angles of less than 10 degrees, can be used to direct fluids on a surface. We have developed a method that coats a glass substrate with either a layer of fluorinated silica nanoparticle or porous polymer monolith to render the surface superhydrophobic. Hydrophilic circular patches (50-250 μm diameter) are produced with laser micromachining by ablating the superhydrophobic coating away, exposing the glass substrate. The hydrophilic patches spontaneously wet when placed in contact with an appropriate liquid and the volume (pL-L) of wetted solvent is contingent upon the patch diameter. The superhydrophobic/hydrophilic array can be placed into conformal contact with a sample and the array samples fluid from the surface. Each patch/spot will be an eventual pixel of the image. Mass spectrometry using DESI or MALDI, with matrix deposition, can then be utilized to analyze chemical signatures on each of the patches to prepare an image of the sample. Surfaces with patterned wettability have the potential to significantly improve sample preparation for mass spectrometric imaging by simplifying matrix deposition and providing a reproducible sample topography to the mass spectrometer.
Spatially Resolved Molecular Analyses of Biological Systems

Localized Probing of Confluent Endothelial Cell Layers Using a Nanophotonic Device

We report a label-free, nanophotonic approach to non-invasively probe real-time changes in endothelial cells resulting from physical or chemical stresses imposed upon them. Adherent cells exhibit a constant, dynamic fluctuation known as cellular micromotion, due to continuous cytoskeletal rearrangement. Impaired cellular micromotion in confluent endothelial layers might serve as an indicator of pathophysiological conditions in cell cultures. Cellular micromotion is an effect that can be recorded by observing the behaviour of an endothelial cell layer under the microscope. However, probing this effect more directly in the cells themselves has the possible advantage of allowing better distinction in shorter times between cell behaviours resulting from different external stimuli.

The read-out for cellular micromotion in our device is forward-scattered laser light, propagated through just a few cells in an endothelial cell layer cultivated on the surface of a combined nanophotonic/microfluidic device. The continuous motion of the cytoskeleton and resultant shifting of other internal cellular components leads to a constant displacement of light-scattering sites, yielding a fluctuating scattered-light signal that can be attributed to cellular micromotion. This contribution will describe a study in which our goal was to gain a better understanding of how cytoskeletal changes affect the signals recorded. Endothelial cells were exposed to compounds known to target specific processes affecting actin, one of the structural proteins making up the cytoskeleton, and forward-light scattering signals were recorded. Confocal images of HUVEC exposed to the same compounds at the same concentrations corroborated the results obtained with the nanocuvette chip. We also report a compact, easy-to-use experimental platform for advanced microfluidic cell culture studies, using our example of few-cell cytometry as an initial demonstration.

Keywords: Bioanalytical, Lab-on-a-Chip/Microfluidics, Light Scattering, Pharmaceutical
Application Code: Pharmaceutical
Methodology Code: Microfluidics/Lab-on-a-Chip
The development of effective therapies for pathologies including cancer and diabetes requires a deep molecular understanding of tissue heterogeneity by advanced omics technologies with spatially resolved measurements. Unfortunately, there are substantial gaps in existing capabilities in terms of sensitivity. For example, a minimum of many thousands of cells have been required for in-depth profiling of proteins in a biological sample. We have recently developed a microfluidic technology, termed nanoPOTS (Nanodroplet Processing in One pot for Trace Samples), in which a robotic platform dispenses cells and reagents into photolithographically patterned nanowell reaction vessels with subnanoliter precision. Sample preparation utilizes a novel workflow that eliminates the need for multiple reaction vessels and cleanup steps to process cellular tissue into purified tryptic peptides. Compared to the typical tens-of-microliter volumes for proteomic sample preparation, the ~200 nL nanowells minimize sample losses to surfaces and maintain elevated sample concentrations for efficient digestion. To date, we have identified >3,000 proteins from just 10 mammalian cells, which is a level of proteome coverage not previously achieved from fewer than thousands of cells, and we have identified a minimum of hundreds of proteins from single mammalian cells. For spatially resolved measurements, laser-capture microdissection serves as an effective means of excising tissues with high spatial resolution from prepared thin sections. We have obtained in-depth proteome profiles from single thin sections of pancreatic islets isolated from healthy and type 1 diabetes donors and are pursuing the in-depth proteome mapping of, e.g., tumor microenvironments with single cell resolution.

**Keywords:** Lab-on-a-Chip/Microfluidics, Liquid Chromatography/Mass Spectroscopy, Proteomics, Ultratrace Ana

**Application Code:** Biomedical

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

In May 2016, the U.S. Food and Drug Administration (FDA) published draft guidance entitled Premarket Tobacco Product Application (PMTA) for Electronic Nicotine Delivery Systems (ENDS). In this guidance, FDA recommends reporting quantities of designated harmful and potentially harmful constituents (HPHCs) in e-cigarette e-liquids and aerosols. The HPHC list comprises potential matrix-related compounds, flavors, nicotine, tobacco-related compounds, container closure leachables, thermal degradation products, and combustion-related compounds found in conventional cigarette smoke. E-cigarettes contain trace levels of many of these constituents due to the inclusion of tobacco-derived nicotine and the thermal degradation products that can be created during aerosol formation. However, HPHCs related to combustion including aromatic amines, volatile organic compounds, and the polycyclic aromatic hydrocarbon benzo[a]pyrene are not likely to be created during aerosol formation due to the relatively low operating temperatures of e-cigarettes. Data and methods for the analytical analysis of commercial and prototype e-cigarette e-liquids and aerosols will be discussed. Specifically, data and methods will be shared for the analysis of carbonyls, tobacco-related compounds, and combustion-related compounds in e-cigarette e-liquids and aerosol. These studies can potentially inform regulatory agencies of the most meaningful analytes to include in regulatory guidance and inform e-liquid and e-cigarette manufacturers of the compounds to monitor for quality control and during stability studies.

### Keywords:
- Aerosols/Particulates, GC-MS, HPLC, Mass Spectrometry

### Application Code:
- Other

### Methodology Code:
- Mass Spectrometry
What is in Your E-Cigarette

Dangerous Flavors: The Effect of Flavoring Additives on Emissions of Toxic Compounds During E-Cigarette Use

The growing popularity of electronic cigarettes (e-cigarettes) highlights the importance of developing product standards that will decrease the potential for short- and long-term adverse health effects. E-cigarettes are available in a wide variety of flavors, one of the main factors that attracts non-smokers and smokers alike. How flavoring compounds in e-cigarette liquids affect chemical composition and toxicity of e-cigarette vapors is practically unknown. Although the current body of research strongly suggests that e-cigarettes are a safer alternative to traditional cigarettes and can help smokers quit, some e-cigarette device/e-liquid combinations are capable of producing toxic aldehydes during vaping. So far, aldehyde formation has been attributed to thermal decomposition of the main components of e-cigarette e-liquids (propylene glycol and glycerol), while the role of flavoring compounds has been ignored. More data about the role of flavoring compounds in aldehyde formation will inform creation of e-cigarette device and liquid regulations to protect individual and public health.

We will present results of our recent studies on the effect of flavoring compounds and e-cigarette operating parameters on the production of toxic carbonyl compounds and carbon monoxide during e-cigarette use. We compare concentrations of these compounds in mainstream e-cigarette aerosols produced by different e-cigarette devices filled with unflavored and various flavored liquids. We will show that flavoring compounds contribute significantly to emissions of the measured toxic compounds. We will also discuss the effects of e-cigarette power, coil temperature, and puff topography (air flow rate and duration). Results of a pilot study with 18 e-cigarette users in which carbonyl concentrations were measured in main stream and exhaled aerosols will also be presented.

Keywords: GC-MS, HPLC, HPLC Detection, Volatile Organic Compounds
Application Code: Consumer Products
Methodology Code: Chemical Methods
What is in Your E-Cigarette

Free Radical Formation in Electronic Cigarette Aerosols

The ever-evolving market of electronic cigarettes (EC) presents a challenge for analyzing and characterizing the harmful products they can produce. We have tested for the presence of reactive, short-lived free radicals in a variety of EC designs by electron paramagnetic resonance spectroscopy (EPR) using the spin-trap phenyl-N-tert-butylnitrone (PBN). Initially, we reported that EC aerosols can deliver high levels of reactive free radicals ($2.5-10.3 \times 10^{13}$ radicals/puff). More recently we have found, using a temperature controlled EC device and novel mechanism for reliably simulating e-cigarette usage conditions, that free radical production from e-liquids was highly temperature and wattage dependent and heavily influenced by the relative propylene glycol (PG) and glycerol (GLY) content. Since flavoring chemicals are a common constituent in e-liquids, we analyzed the free radical production from 49 popular nicotine-free e-liquids flavors and observed that nearly half modulated free radical production as compared to a base of PG:VG (60:40) alone. The specific flavor chemicals in each e-liquid flavor were identified using gas chromatography mass spectroscopy (GCMS) and their abundance was correlated with radical production from their parent e-liquid flavor. When the flavor chemicals with the strongest positive or negative correlations were analyzed for their individual impact on free radical generation, several, including dipentene, ethyl maltol, citral, linalool, or piperonal were found to promote free radical formation, while others, including ethyl vanillin, inhibited radical formation. Overall, radical production was associated with increases in EC aerosol-induced oxidation of biologically relevant lipids. Altogether, these findings suggest that free radical production by EC is highly dependent on the design and composition of the EC device and e-liquid and may represent a potential harm to the EC user.

Abstract Text

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Keywords: Environmental, Flavor/Essential Oil, GC-MS
Application Code: Environmental
Methodology Code: Magnetic Resonance
Hookah use is increasingly prevalent among college age students. The health impacts of hookah or waterpipe tobacco smoke (WTS), however, are only poorly understood and potentially hampered by the limitations of approaches comparing WTS to secondhand smoke from cigarettes. The combination of shisha as a very different matrix from cigarette tobacco and indirect heating versus combustion, however, may present unknown health hazards. In particular, we are focused on the potential physical toxicity associated with the inherent physical properties of particles in WTS. In previous studies, we have found that the height of the waterpipe or hookah impacts the distribution and concentration of the particulate component of waterpipe tobacco smoke (WTS). Here, we present the results of systematic investigations into the contribution of different components within shisha as well as the use of charcoal or an electronic heating source to the distribution and concentration of the particulate component of WTS under different smoking regimes. Additionally, the results from preliminary investigations of different hose lengths will be presented.

Keywords: Aerosols/Particulates, Consumer Products
Application Code: Consumer Products
Methodology Code: Physical Measurements
Electronic cigarettes ("e-cigarettes") aerosolize a solution of nicotine and other additives by application of power to a heating coil. Food flavorings are forming an increasingly large portion of the "other additives" category, due to the appeal of fruity and candy-tasting flavors to the major e-cigarette user group — youth and young adults. Another trend among e-cigarette consumers is the use of devices with variable power settings. The rationale is that by using higher power settings, one can get a bigger "hit" of nicotine and/or flavoring per puff. Due to the rapid pace at which e-liquids and e-cigarettes are evolving, this assumption, and even the fundamental question of the efficiency of transfer of flavorings from liquid to aerosol, have yet to be answered rigorously. In this work, e-liquids which have been shown to contain some of the most common e-liquid flavorings were aerosolized using a variable-power device and the major flavors and nicotine were quantified in the aerosol as a function of power setting. In addition, the same compounds were quantified in the liquid so that transfer efficiencies could be calculated.

Keywords: Aerosols/Particulates, Characterization, Instrumentation, Mass Spectrometry
Application Code: Consumer Products
Methodology Code: Mass Spectrometry
We are studying the means by which enzymatic biosensors degrade once implanted in the brain. While enzymatic biosensors are an effective method for monitoring neurotransmitter release in near-real time, their lifetime during in vivo studies requires better understanding and optimization for long term use. Current methods of neurotransmitter detection rely on the electrochemical oxidation of the analyte of interest at the electrode surface. For neurotransmitters that are not electrochemically active, the immobilization of an enzyme layer onto the electrode surface is necessary to generate an electrochemically active molecule (typically hydrogen peroxide) in the presence of the analyte of interest. However, this enzyme layer only lasts approximately two weeks after implantation in the brain, rendering long-term use of these biosensors for in vivo studies ineffective.

This talk will focus on how exposure of enzymatic devices to solutions designed to mimic certain chemical components present in the brain affects device lifetime. A flexible, polymer-based microelectrode array was fabricated, onto which glutamate oxidase was immobilized. The biosensors were exposed to a variety of environmental parameters, with device sensitivity to glutamate measured and tracked weekly. To better stabilize the enzyme against degradation in the brain, a proprietary nanoparticle called nanoScyl was incorporated into the immobilized enzyme layer. nanoScyl was designed to encapsulate and protect the enzyme from degradation. We have demonstrated initial improved enzyme stability with nanoScyl by measuring the sensitivity of devices onto which nanoScyl and glutamate oxidase was immobilized. With improved immobilization methods, nanoScyl has the potential to improve enzyme lifetime for chronic use in vivo.

Keywords: Biosensors, Chemically Modified Electrodes, Neurochemistry
Application Code: Neurochemistry
Methodology Code: Sensors
Single-cell amperometry is a powerful technique for measuring neurochemical release from cells and elucidating the biochemical mechanisms of these release events. Current methodologies for single-cell amperometry include manual fabrication and positioning of carbon-fiber disk electrodes above single adherent cells. We have designed, fabricated, characterized, and implemented a microfluidic chip that is capable of capturing single cells on a polymer microelectrode and measuring chemical release from that cell amperometrically. The device is fabricated in two parts: (i) PEDOT:tosylate electrodes are patterned using standard photolithography techniques on one part of the chip and (ii) the fluidics, including the cell-capture channel, are made by injection molding on a second part of the chip. The two halves are then permanently bonded to create an all-polymer device that can be used to measure chemical release from single cells. By using fluidics to direct the cell to a stationary electrode, this design circumvents the traditional methodology of bringing the electrode to the cell. A common model system for dopamine vesicle release, PC-12 cells, was investigated using this new device. Cells were successfully captured at the electrode surface using software-controlled suction and exocytosis events were recorded. Further, we used pharmacological challenges such as Lithium, L-DOPA, and PACAP, along with comparisons to carbon-fiber electrodes to validate the quantitative characteristics of this measurement platform. This novel chip design ultimately provides a more efficient and controlled method to perform single-cell amperometry.
Overcoming Challenges of In Vivo Neurotransmitter Detection Using Aptamer Field-Effect Transistors

In vivo semiconducting biosensors have traditionally suffered from the inability to circumvent Debye length limitations. The Debye length is the distance beyond the semiconducting channel surface of biosensors where changes in local electric fields affect the distribution of free charge carriers in the channel. To monitor conductance change in vivo where physiological conditions are highly ionic, the Debye length is less than 1 nm. We tackle this challenge by coupling the molecular recognition properties of rationally designed, chemically synthesized DNA sequences, termed aptamers, with direct signal detection via field-effect transistors (FETs). Aptamers can be designed to selectively recognize specific targets, and for optimized signal transduction, and in vivo stability. We monitor changes in neurotransmitter concentrations via binding-induced aptamer conformational changes that are transduced into electrical signals. Reversible binding of target neurotransmitters such as serotonin and dopamine by specific aptamers are hypothesized to shift electric potentials in close proximity to FET surfaces, resulting in conductance changes. Data derived from spectroscopic methods, such as circular dichroism and surface-enhanced Raman spectroscopy, support our hypotheses for aptamer detection mechanisms. Serotonin- and dopamine-specific aptamer FETs have high sensitivity for their targets with unprecedented femtomolar detection limits. These devices retain functionality in full ionic strength biological fluids including artificial cerebrospinal fluid. We have conducted measurements ex vivo in brain tissue homogenates of Tph2 knockout mice lacking serotonin to test device responses upon controlled addition of serotonin while assessing biofouling. Through systematic delineation of the mechanistic aspects of aptamer-FET sensing to enable rational optimization of these biosensors, we envision pushing the limits of in vivo neurotransmitter detection beyond conventional biosensing.

Keywords: Bioanalytical, Biosensors, Neurochemistry, Nucleic Acids
Application Code: Neurochemistry
Methodology Code: Sensors
Carbon nanomaterials have been widely used as electrode materials for the detection of neurotransmitters due to their high bio-compatibility, fast electron transfer, and abundant electroactive sites. Great focus was put on the applications of new carbon materials and designs; however, the surface roughness significantly affects the detection capability of neurotransmitters. And the lack of understanding on the effect of the electrode surface roughness brings variance of the electrode performances fabricated by different research groups. Venton lab utilizes electrodes with well-controlled surface roughness using fast scan cyclic voltammetry (FSCV), including the antistatic gun treated carbon nanotube (CNT) yarn microelectrodes, cavity carbon nanopipette electrodes (CNP), and 3D printed microelectrodes with controllable surface roughness. The antistatic gun treated CNT yarn microelectrodes have an increased surface roughness and identical amount of functional groups compared to the unmodified CNT yarn microelectrode. The experimental and simulation results demonstrate the rough surface (> 1900nm) causes the frequency independence property using FSCV. Furthermore, we have used the CNPs with a cavity-shaped tip (~500 nm in diameter) to mimic a unit of the rough surface. The cavity geometry traps neurotransmitters in the crevice and improves the detection sensitivity. In addition, the cavity geometry boosts the electrical field at the electrode tip, which improves the selectivity to cations such as dopamine over ascorbic acid and uric acid. These studies contribute to a more comprehensive understanding of the correlation between surface roughness and electrochemical performance.

Keywords: Biomedical, Electrochemistry, Electrodes, Voltammetry
Application Code: Neurochemistry
Methodology Code: Electrochemistry
### Abstract Text

Continued evolution of neurochemical monitoring is necessary to advance our understanding of brain-behavior relationships and the neurotransmitter basis of neuropathologies. To this end, we are pursuing two integrated circuit (IC) approaches implementing fast-scan cyclic voltammetry, which when combined with a microelectrode affords high temporal, spatial, and chemical resolution to neurochemical monitoring. The first approach resulted in development of the “neurochemostat” IC for regulating brain extracellular dopamine (DA). This novel IC combined wireless operation, FSCV, on-the-fly principle component regression (PCR), and feedback-controlled microstimulation. PCR is a chemometrics approach that has become the standard tool for resolving DA from multianalyte FSCV signals. Proof-of-principle was demonstrated in anesthetized rats by the maintenance of electrically evoked levels of striatal DA between two user-set thresholds. During operation and in real time, DA was resolved from pH changes and background current drift. The goal of the second approach is a multichannel microdevice supporting high-site density distributed neurochemical recording using multielectrode arrays (MEAs). The IC strategy is well suited for scaling up recording channels while maintaining a small form-factor, but data transmission rates limit simultaneous operation to 4 channels in our wireless ICs. To increase functional channel number, we are developing compressive FSCV (C–FSCV), which combines FSCV and compressive sensing theory for real-time data compression. In C–FSCV, signals are compressively sampled with a sensing matrix and then algorithmically reconstructed using a sparsifying dictionary matrix. Proof-of-principle for C–FSCV was demonstrated by the compression and high-fidelity reconstruction of electrically evoked DA signals pre-recorded in the striatum. Success with compression factors as high as ~5 bode well for future development of a wireless C–FSCV microdevice supporting MEAs.

**Keywords:** Bioanalytical, Biomedical, Neurochemistry, Voltammetry

**Application Code:** Biomedical

**Methodology Code:** Chemical Methods
Dopamine is an important neurochemical involved in governing mood, habit formation, and many other critical behaviors. Dopamine neurotransmission has traditionally been measured from a single brain site with a single channel sensor, which has limited our ability to understand its spatial variation across brain regions. Multichannel sensors were fabricated to probe neurotransmission from multiple sites (up to 16 channels) of the brain. This microfabricated carbon fiber array provides 8 neurochemical sensors spaced 250 μm apart. Each sensor is 4 – 6 mm long to target deep striatal brain targets. Individual sensors were designed to provide cellular-scale footprints (diameter of < 10 μm) to suppress neuroinflammatory processes that can obscure accurate neurochemical detection. The sensors did not induce visible changes in astrocytic or microglial expression around implanted sites when evaluated with immunohistochemistry. This contrasts with traditional microelectrodes that were shown to heighten expression of these inflammatory markers up to 100 – 500 μm away from implanted sites. Open-source instrumentation was constructed that allows voltammetric recording from up to 16 probes or 2 implanted arrays in order to monitor synchronously neurochemicals from multiple implanted sensors. The recording platform provides similar dynamic range (±2000 nA), sampling resolution (214 samples per scan or 25 kHz), and noise (<0.1 nA) to traditional cyclic voltammetry instruments. The arrays were implanted into the striatum of rats to demonstrate the heterogeneous dopamine release induced by stimulation of the medial forebrain bundle, axonal dopamine-containing fibers that project to the striatum. We also evaluated how these signals are modulated by dopamine-selective drugs across brain sites.

Keywords: Array Detectors, Electrochemistry, Microelectrode, Neurochemistry
Application Code: Neurochemistry
Methodology Code: Sensors
Pushing the Limits of In Vivo Neurotransmitter Detection

Hydrogen Peroxide, Dopamine, and Serotonin: Overlapping Chemical Systems in the Rat Contribute to the Control of Dyskinetic Movements During Chronic L-DOPA Treatment for Parkinson’s Disease

Parkinson’s disease (PD) is characterized by the slow degeneration of dopamine (DA) cells that are critically involved in motor control. Oxidative stress is thought to contribute to the initiation and progression of motor dysfunction, but the mechanisms by which this occurs remain unclear. Furthermore, the effects of the gold standard therapy, L-DOPA, are controversial. Some studies suggest that L-DOPA treatment potentiates damage to dopaminergic neurons due to oxidative stress resulting from radical and quinone metabolites. Other studies indicate that L-DOPA can decrease lipid peroxidation and scavenge reactive oxygen species. Moreover, raphestriatal serotonin (5-HT) neurons may compensate for the progressive loss of nigrostriatal DA neurons by converting and releasing DA during L-DOPA replacement therapy. DA released from 5-HT terminals would be unfettered due to a lack of DA-sensitive autoreceptors, and dramatically fluctuating DA concentrations in the striatum may contribute to the development of L-DOPA-induced dyskinesia. This work investigates these questions with voltammetric measurements of real-time hydrogen peroxide and DA dynamics at single micron-scale recording sites in a rat model of PD (unilateral 6-OHDA lesion) over several weeks of L-DOPA administration. Chemical fluctuations in striatum are correlated with behavioral abnormalities that develop over the course of treatment. Citalopram, a 5-HT reuptake inhibitor, attenuates both the chemical dynamics and the abnormal movements when administered before L-DOPA, demonstrating that 5-HT terminals significantly influence the striatal environment in this system. These studies promise to aid in our understanding of chemical mechanisms affecting the development and treatment of dyskinesias in the management of PD.

Keywords: Electrochemistry, Microelectrode, Neurochemistry, Voltammetry
Application Code: Neurochemistry
Methodology Code: Electrochemistry
Dopamine (DA) is a monoamine neurotransmitter responsible for the maintenance of a variety of vital life functions. In vivo DA signaling occurs over multiple time scales. Phasic DA release induces sub-second transient concentration fluctuations whereas tonic firing maintains local basal DA concentration and is responsible for long-term DA concentration changes occurring over minutes to hours. Due to the complex, multifaceted nature of DA signaling, analytical sensing technology must be capable of recording DA from multiple locations and over multiple timescales. Decades of research has focused on improving in vivo detection capabilities for sub-second phasic DA, but the accurate detection of absolute resting DA levels has proven challenging. We have developed a poly (3,4 ethylene dioxythiophene) (PEDOT)-based nanocomposite coating that exhibits excellent DA sensing capabilities for resting DA. PEDOT/functionalized carbon nanotube coated CFEs are capable of directly measuring resting DA levels using square wave voltammetry with high sensitivity and selectivity. The sensitivity for resting DA increases with PEDOT/fCNT coating thickness, with the optimal condition exhibiting a 4700% increase in sensitivity for resting DA as compared to bare CFEs, a sub-50 nM lower limit of detection for DA and stable continuous recording capabilities over multiple hours. PEDOT/fCNT coated CFEs are capable of directly measuring the absolute in vivo basal DA concentration as well as the pharmacologically induced increase in tonic DA caused by the dopamine transporter inhibitor, nomifensine. This study introduces an exciting new electrode coating/in vivo voltammetric methodology that shows great promise for the direct detection of tonic DA.

Funding provided by: NIH R01NS062019

Keywords: Electrochemistry, Neurochemistry, Polymers & Plastics, Voltammetry

Application Code: Neurochemistry

Methodology Code: Electrochemistry
In the Boika group, we are developing analytical methodologies based on the use of electrokinetic phenomena to improve the stochastic electrochemical detection of various analytes. The phenomena of interest include dielectrophoresis (DEP) and electrothermal fluid flow (ETF). These phenomena are induced in an electrolyte solution by applying a high frequency (~100 MHz) alternating current (ac) waveform between a counter and a working electrode. The investigated analytes include metal nanoparticles such as Ag and Pt, and E. coli bacteria. Our results indicate that the electrochemical detection of these analytes is improved significantly by the presence of the aforementioned electrokinetic phenomena. The DEP and ETF manipulation of the analytes leads to their effective preconcentration at the indicator electrode surface and is also accompanied by an improved faradaic response. It is suggested that the methodology being developed will allow for the detection of ultralow concentrations of many other important bioanalytes.

Keywords: Electrochemistry, Method Development, Microelectrode
Application Code: Bioanalytical
Methodology Code: Electrochemistry
The use of particles for the controlled delivery of cargo, such as a drug or growth factor, represents an exciting way to target delivery of active molecules to cells or tissue. With drug delivery this mechanism provides a way to maintain drug concentration over longer periods and can reduce side effects arising from the high drug doses required for non-targeted delivery. In terms of \textit{in vitro} tissue culture, particles can overcome the limitations associated with directly supplementing growth factors to cell cultures, allowing controlled release. The common factor across these areas is the requirement for cargo delivery where it’s needed and when desired.

Novel synthetic polymer particles are highly suited as delivery vehicles for their advantages including adaptable surface chemistry, high cargo loading and prolonged circulation. Release can be controlled by engineering particles that respond to environmental stimuli including mimics of intracellular environments (1), temperature and the presence of degradative enzymes (2, 3). Within the Stevens group, particles have been tailored to release cargo in response to selected enzymes.

Currently, particle release is monitored via fluorescent cargo and can involve complex sample preparation for measurement. This can prove difficult for quantitative measurements, provides discrete time points, can influence cargo release and may not be suitable across platforms. Being able to monitor release continuously is beneficial.

In this work we use microdialysis as a non-destructive sampling technique enabling the real-time monitoring of cargo release. This study investigates cargo release from different particles under different environmental conditions. Results will be compared using fluorescent and electrochemical particle loads.

Bioanalytical Electrochemistry

**Abstract Title**

Coupling PEDOT-CNT Coated Microelectrodes with Electrochemical Impedance Spectroscopy to Measure Brain Extracellular Space Morphology

**Primary Author**

Jenna DeVivo
University of Pittsburgh

**Co-Author(s)**

Adrian C. Michael, Elaine M. Robbins, Ian M. Taylor, Stephen G. Weber, Tracy X. Cui, Yangguang Ou

**Abstract Text**

To understand the transport of analytes in the brain, it is important to understand the various processes that affect the fate of the analyte, including reuptake, degradation, and diffusion. The geometry of the extracellular space (ECS) also plays a role, as the tortuous nature of the ECS affects the effective diffusion coefficient. The ECS can be characterized by a unitless number from geology called the formation factor (ff), which is the ratio of porosity (φ) to tortuosity (T) and is defined as φ / T^2. These values have been previously measured in the rat brain using methods such as real-time iontophoresis of tetramethylammonium ions. We aim to measure spatial and temporal changes in ff with a less invasive, analyte-free method that requires only a single probe. Our method couples 7-μm (diameter) x 400-μm (length) carbon-fiber microelectrodes coated with poly(3,4-ethylenedioxythiophene)-carbon nanotubes (PEDOT-CNT) with electrochemical impedance spectroscopy (EIS). We apply a small AC excitation potential to the microelectrode (10 mV) and monitor the cell response in the 1-100kHz regime. We use a coating surface charge density of 15 mC/cm^2, which corresponds to a capacitance of 0.34-0.45 mF/cm^2. We also developed an equivalent circuit model to fit the EIS spectra and extract the resistance of the surrounding medium. Using a calibration curve generated in vitro for different concentrations of KCl, we can determine the effective conductivity of the surrounding medium. With this method, we reproducibly measured the spatial changes in ff as the microelectrode is lowered from the cortex to the corpus callosum to the ventricles (see figure). Interestingly, the ff in the ventricles is close to 1, with a value of 0.84±0.1 (SEM, n = 3). We also measured the temporal changes in ff over 30 min as a result of a needle pinprick in the cortex.

**Keywords:** Bioanalytical, Electrochemistry, Electrode Surfaces, Microelectrode

**Application Code:** Bioanalytical

**Methodology Code:** Electrochemistry
We have reported earlier about the interaction of hexammine ruthenium(III) (RuHex) and hexammine cobalt(III) (CoHex) with DNA on gold electrodes by electrochemical quartz crystal microbalance (EQCM). These complexes interact with DNA both electrostatically and by formation of hydrogen bonds. We have observed a large frequency change of ca. 30 Hz (Fig. 1A, B) upon 450 mV potential jumps that could not be explained by a loss or gain of complex cations or counter anions. The EQCM response was 20 Hz with CoHex. It occurred only when a proper potential jump around the redox potential of RuHex or CoHex was applied. For CoHex, it took 30 seconds to reach a new stable frequency in contrast to only 240 ms in case of RuHex. Without RuHex and CoHex, a very small frequency response (4 Hz) occurred within 80 ms. Viscoelasticity of the DNA-SAM may be influenced by the interaction with the different complex ions. CoHex is known to influence the secondary and tertiary structure of DNA forming intermolecular bridges between strands.[1] This mechanism was supported by the observed major H/D kinetic isotope effects with CoHex that were found for voltammetry and EQCM studies in deuterium oxide-based electrolytes (Fig. 1C, D). With RuHex, this H/D-effect was significantly smaller. Here, we report on the effects of DNA surface coverage upon the frequency shift. It seems that both redox switching of EQCM response and H/D kinetic isotope effects can shed light on the complicated structure of gold-thiol-based layers consisting of DNA and backfill molecules such as mercaptohexanol.

References:
There has been increased interest in the development of fast, sensitive, and cost-effective DNA sensing devices for sequence-specific detection of clinically, environmentally, and government security-relevant nucleic acid targets. Recent observations have shown DNA immobilization at the surface of a gold electrode play a vital role in the performance of any DNA-based sensor. Here, we conducted a distance-dependence study via square wave voltammetry (SWV), as a function of pulse frequency. Using methylene blue (MB) as a redox tag attached to DNA, we first verified appropriate lengths of a spacer region in the DNA sequence toward a hairpin-based switch sensor design. Based on these results, we then engineered a switch sensor using a novel, bistable DNA hairpin structure that is capable of nucleic acid (DNA or RNA) detection. Our switch utilizes a strand displacement mechanism that holds the MB-DNA further from the surface until a target strand is hybridized, at which time the MB-DNA is hybridized near the electrode. Our results confirm that as the concentration of the target increases, the current increases. As SWV frequency is increased, the sensor changes from a diffusion-limited, square-root dependence at low concentrations of target to a more linear, adsorbed-like dependence at high concentrations of target (Figure), further confirming the hairpin switching mechanism. For DNA targets, the sensor shows a concentration-dependent response with a limit of detection of 4.4 nM, showing promise for future applications to other nucleic acids such as messenger RNA (mRNA) and micro RNA (miRNA). We are currently verifying the sensor for mRNA and miRNA that correlate to diabetes research such as miRNA-375 and insulin-1 mRNA.

**Abstract Text**

There has been increased interest in the development of fast, sensitive, and cost-effective DNA sensing devices for sequence-specific detection of clinically, environmentally, and government security-relevant nucleic acid targets. Recent observations have shown DNA immobilization at the surface of a gold electrode play a vital role in the performance of any DNA-based sensor. Here, we conducted a distance-dependence study via square wave voltammetry (SWV), as a function of pulse frequency. Using methylene blue (MB) as a redox tag attached to DNA, we first verified appropriate lengths of a spacer region in the DNA sequence toward a hairpin-based switch sensor design. Based on these results, we then engineered a switch sensor using a novel, bistable DNA hairpin structure that is capable of nucleic acid (DNA or RNA) detection. Our switch utilizes a strand displacement mechanism that holds the MB-DNA further from the surface until a target strand is hybridized, at which time the MB-DNA is hybridized near the electrode. Our results confirm that as the concentration of the target increases, the current increases. As SWV frequency is increased, the sensor changes from a diffusion-limited, square-root dependence at low concentrations of target to a more linear, adsorbed-like dependence at high concentrations of target (Figure), further confirming the hairpin switching mechanism. For DNA targets, the sensor shows a concentration-dependent response with a limit of detection of 4.4 nM, showing promise for future applications to other nucleic acids such as messenger RNA (mRNA) and micro RNA (miRNA). We are currently verifying the sensor for mRNA and miRNA that correlate to diabetes research such as miRNA-375 and insulin-1 mRNA.
In recent years there has been considerable interest in the development of continuous physiological monitoring technologies for assessing physical performance in athletes. Real-time sweat analysis is of particular interest as it can provide a non-invasive means of measuring key analytes and hence provide valuable information on a person’s physiological state and hydration levels. This would be particularly useful for athletes as a 2% loss in body weight due to dehydration can reduce athletic performance capacity by 20%, therefore controlling hydration could enhance performance.

We are developing a wearable analysis patch that quantifies key sweat markers in real time. The novel platform incorporates a semi-permeable membrane that will be perfused to collect key analytes from sweat. This sampling patch will be coupled to a flow cell containing microelectrode-based amperometric metabolite biosensors [1, 2] and miniature ion-selective electrodes to measure changes in levels of these key analytes. The wearable platform will also incorporate wireless battery-powered control electronics, which link to a laptop or tablet via Bluetooth to visualise trends in real time.

The novel device will be tested using a computer-controlled microfluidic flow chamber, allowing us to mimic dynamic molecular changes in sweat. Proof-of-concept non-invasive sweat measurements will be presented from exercising athletes during and after various levels of exercise to quantify the effect of exercise and exhaustion on sweat markers including ions, glucose and lactate.

References:
Bioanalytical Electrochemistry

Small-Volume Thermoplastic Electrochemical Array Sensors

A major challenge in electrochemistry is single molecule analysis. One approach to achieve this goal is performing analysis in very small volumes, but these systems are difficult to fabricate from low-cost materials such as carbon electrodes. Carbon electrodes are of interest for their biocompatibility and large potential window. Thermoplastic electrodes (TPEs) recently developed by our group offer easy and low-cost fabrication comparable to screen printing, but exhibit significantly better electrocatalytic activity. Additionally, TPEs are highly conductive and can be fabricated into small and complex geometries. Small volume array designs can eliminate concerns about mass transport, sample size limitations, and electrode crosstalk. In this work, stereolithography (SLA) 3D printing is used for the rapid prototyping of micro-vial TPE electrode arrays (Figure 1a). The electrodes are characterized and optimized with voltammetry and impedance spectroscopy experiments using established redox couples. 3D printing, optical profilometry, and scanning electron microscopy (SEM) are used to optimize the fabrication of micro-vial arrays. The TPE electrodes are incorporated into the micro-vial devices to study the effects of volume on performance (Figure 1b). Lastly, these 3D printed micro-vial TPE arrays can be functionalized for specific biomolecules for selective, ultra-sensitive, small-volume analysis.

Work funded by the NSF.

Keywords: Biosensors, Electrochemistry, Electrodes, Sensors
Application Code: Bioanalytical
Methodology Code: Electrochemistry
The use of paper-based analytical devices as an alternative to traditional benchtop methods has garnered significant interest due to their low cost, simple fabrication, and applicability for point-of-care testing. Paper sensors, particularly when coupled with electrochemical detection, have the potential to substantially improve assay time and detection limits for many applications. To date, electrochemical paper-based analytical devices (ePADs) have relied almost exclusively on single electrode detection, limiting potential gains in sensitivity and/or selectivity achievable by multiple electrodes. Herein we describe incorporation of an interdigitated electrode array (IDA) into an ePAD. Generation-collection experiments can be performed using this IDA where the first electrode in the array oxidizes the analyte, the second reduces it, and the process is then repeated across the entire array to provide enhanced signal and chemical selectivity. The IDA was fabricated using graphite and cyclic olefin copolymer via a simple solvent-assisted method, and coupled to a fan-shaped paper device whose geometry allows for a quasi-stationary flow of solution. The electrodes show an improved electrochemical activity over typical screen-printed carbon electrodes and have a good mechanical stability within the flow system. Up to 46% collection efficiency was achieved using a multi-layer paper device, which is higher than previously reported flow-based IDA operated at similar flow rates. Analyte conversion at the electrodes was improved by increasing the number of electrodes and decreasing both the height of the flow channel and pore radius of the paper substrate. With the improved sensitivity and selectivity, the IDA-ePAD can be employed for detecting biologically relevant molecules with low detection limits.

**Keywords:** Bioanalytical, Electrochemistry, Electrodes, Paper/Pulp

**Application Code:** Bioanalytical

**Methodology Code:** Electrochemistry
Bioanalytical Sensors  
Photonic Crystal Carbohydrate Hydrogel Sensors for Sensitive and Selective Detection of Lectin Proteins

Zhongyu Cai  
University of Pittsburgh

Aniruddha Sasmal, Sanford A. Asher, Xinyu Liu

We report the development of carbohydrate containing responsive hydrogel sensing materials for the selective detection of lectin proteins. The copolymerization of a vinyl linked carbohydrate monomer with acrylamide and acrylic acid forms a carbohydrate hydrogel that shows specific “multivalent” binding to lectin proteins. The multivalent interactions can greatly enhance the sensitivity and response of the resulting hydrogel sensing materials. In this study, the resulting carbohydrate hydrogels are attached to 2-D photonic crystals (PCs) that brightly diffract visible light. This diffraction provides an optical readout that sensitively monitors the hydrogel volume. We utilize lactose, galactose and mannose containing hydrogels to fabricate a series of 2-D PC sensors that show strong selective binding to the lectin proteins ricin, jacalin and concanavalin A (Con A). This binding causes a carbohydrate hydrogel shrinkage which significantly shifts the diffraction wavelength. The resulting 2-D PC sensors can selectively detect the lectin proteins ricin, jacalin and Con A. These unoptimized 2-D PC hydrogel sensors show a limit of detection (LoD) of 7.5 x 10^{-8} M for ricin, a LoD of 2.3 x 10^{-7} M for jacalin, and a LoD of 3.8 x 10^{-8} M for Con A, respectively. The combination of this facile copolymerization method and the 2-D PC sensing platform enable a number of other carbohydrate sensors for the selective detection of other lectin proteins.

This work was financially supported by HDTRA under Grant No. 1-15-1-0038.

Keywords: Bioanalytical, Biosensors, Carbohydrates, Protein  
Application Code: Bioanalytical  
Methodology Code: Sensors
Enzymatic activatable fluorogenic molecular probes development holds great significance in signaling cancer-associated species, unraveling the mystery of cancer onset and progression, as well as their potential use in fluorescence-guided cytoreductive surgery. Human NAD(P)H:quinone oxidoreductase isozyme I (hNQO1), targeted in this research, has upregulated expression in a multitude of solid tumors and close association with tumorigenesis and metastasis processes, which makes it a promising biomarker to develop fluorescent probes. A near infrared (NIR), wavelength-shiftable fluorescent probe Q3STCy was successfully developed to selectively and rapidly report hNQO1 activity in cellulo, in 3-dimensional colorectal tumor mimics, and in a xenograft mouse model. Confocal cellular imaging demonstrated its capability with high integrity to differentiate tumor cell lines with different hNQO1 expression levels. Its capability in imaging colorectal multicellular spheroids (1 mm diameter) with spatially heterogeneous hNQO1 activities and detecting human ovarian cancer-derived metastases (0.5 mm dimension) in xenograft mouse models holds much potential in applications that range from drug development to cancer imaging.
Potassium is one of the most abundant cations found in the body. It is of particular clinical interest due to its implications for cancer treatment. It has been shown that the necrotic core within a tumor can induce local, 5 to 10 fold increases in the potassium concentration, which has been shown to inhibit immune cell function. However, the development of this hyperkalemic state, including rate, extent, and distribution, are not characterized and have strong therapeutic implications. We developed a potassium nanosensor that is capable of performing as a functional photoacoustic contrast agent. The optode is based on the principle of Donnan exclusion in which a pH sensitive dye deprotonates when a potassium ionophore chelates a potassium ion. Our sensor is multimodal, and has been calibrated for absorption, fluorescence, and photoacoustic read-out modes over biologically relevant potassium concentrations: 1mM to 100mM for photoacoustics and 20mM to 1M for fluorescence. The sensor shows exquisite selectivity over common interfering ions, such as sodium, magnesium, and calcium. By introducing primary amines to the surface of the sensor, we have been able to functionalize and target the sensor, both passively through the Enhanced Permeability and Retention effect and actively via the attachment of the tumor-homing F3 peptide. The nanosensor has been applied to ex vivo samples, where it was demonstrated that tumor samples have a relatively large concentrations of potassium compared to healthy tissue.
The development of nanostructured probes with unique optical, electronic, and magnetic properties has opened up new possibilities for molecular imaging and spectroscopic detection of specific targets in biomedical and environmental applications. Surface enhanced Raman scattering (SERS) that allows for detecting fingerprint vibrational spectra of single molecules has emerged as a powerful technique for ultrasensitive detection. Plasmonic metal nanostructures with confined electromagnetic field arising from localized surface plasmon resonance (LSPR) are compelling SERS substrates under intense research. In particular, nanogaps formed between closely arrangement plasmonic nanostructures are highly efficient SERS hotspots. This talk summarize our recent work in tailoring the nanogap structures in core-shell metal nanoparticles and two-dimensional arrays of nanostructures at the oil-water interfaces. The synthesis of nanogapped nanoparticles takes advantage of nanoparticle-templated self-assembly of amphiphilic block copolymers and redox-active polymer coating for tailored nanogap engineering for greatly improved SERS activity. The formation of two-dimensional arrays of nanostructures is driven by the interfacial activity of nanoscale particles. We will also discuss the strategies to precisely locate Raman reporters at the nanogap hotspots. The tailored plasmonic structures that allow for enormous SERS signal amplification were applied to detection a range of molecular and cellular targets.
Phospholipid vesicles have been used to co-encapsulate and protect molecular components (e.g., proteins, enzymes, and reporter fluorophores) from the potentially destructive intracellular environment to make nanometer-sized sensors for the detection and measurement of analytes within cells with high spatial and temporal resolution. These carrier vesicles have been further improved by increasing their stability via polymerization techniques, and by introducing size selective pores to improve membrane transport. The resulting porous phospholipid nanoshells (PPNs) are robust enough to withstand experimental conditions, protect encapsulated sensor components from the intracellular environment, and allow analyte molecules to pass through the vesicle membrane without leaching encapsulated sensor molecules. The loading of PPN-based sensors into cells presents a separate challenge, as passive uptake of unmodified PPNs is low and many loading methods are disruptive and damaging to cells. Furthermore, PPN sensors must be freely available within the cellular volume rather than sequestered in endosomes or adsorbed to the cell membrane. To address these challenges, PPN sensor introduction methods including the use of cell-penetrating peptides, vesicle surface charge augmentation, and picoinjection have been explored and were evaluated with respect to loading efficiency, bioavailability, and cell viability in pancreatic cell lines. Sensor loading and location within/on cells was assessed by confocal microscopy, while cell viability and cellular function for each loading protocol for all methods was studied by assays such as MTT and intracellular Ca2+ monitoring using Ca2+ sensitive fluorescent probes to determine which methods yield maximum signal from loaded sensors with minimum decrease in cell viability and function.

This research is funded by NIH GM116946

Keywords: Bioanalytical, Fluorescence, Lipids, Sensors
Application Code: Bioanalytical
Methodology Code: Sensors
In numerous biosensor platforms, fluorescent transducers are used as analyte-selective reporters or for normalization purposes. Lipid vesicles are often used in biosensor platforms to mimic the function and/or composition of bilayer membranes in cells, expanding the variety of receptors that can be integrated. Fluorescent transducers can be doped directly into lipid membranes or via loading into the vesicle interior. Fundamental questions in the vesicle formation and doping processes remain unanswered including understanding the fluorescent lipid distribution and uniformity of doping within single vesicle populations, as well as co-loading characteristics when multiple sensor species are integrated into the sensor architecture. The properties are difficult to discern using traditional flow cytometry which is limited to particles greater than 500 nm. Microscopic comparisons are problematic due to the small size of the vesicles which are smaller than the diffraction limit. A nanoflow cytometer was constructed with a sheath flow cuvette and laser induced fluorescence detection to evaluate the uniformity of fluorophore distributions for single and multiple fluorophore functionalized vesicle, as well as a direct comparison of dye uniformity for membrane associated versus aqueous encapsulated fluorophore uniformity. The detection limit of fluorescein and other fluorophores was optimized by manipulation of the relative flow rates of the sample stream and sheath stream. The information provided by the developed nano flow cytometer will greatly enhance our understanding of vesicle distributions and will enable the rational design of higher efficiency sensors.
Pyocyanin is a virulence factor exclusively secreted as a secondary metabolite by the opportunistic human pathogen \textit{Pseudomonas aeruginosa}. Fast and direct detection of pyocyanin is of importance as it could provide fundamental insights regarding \textit{P. aeruginosa}'s virulence mechanisms. Here, we demonstrate an electrochemical-sensing platform of redox-active pyocyanin using transparent carbon ultramicroelectrode arrays (T-CUAs). We quantified pyocyanin concentrations on various types of T-CUA electrodes using square-wave voltammetry to determine limits of detection (LODs) and linear dynamic ranges (LDRs). LODs and LDRs fall within the micromolar range for a variety of \textit{in vitro} and \textit{in vivo} cellular environments and offer promise of the application of T-CUAs as sensing devices for the quantitative study of biotoxins, bacterial group behavior phenotypes, and pathogenesis. We also demonstrate successful use of T-CUAs for electrochemical detection of pyocyanin secreted from \textit{P. aeruginosa} strains while optically imaging the cells through the transparent electrodes. Secreted pyocyanin levels from two virulent bacterial strains, clinical PA11 and wild-type PA14, were measured. Finally, we present real-time electrochemical monitoring of pyocyanin using T-CUAs while optically and fluorescently analyzing aggregate formation of various clinical and laboratory bacterial strains.
The development of optical pH sensors for measuring intracellular pH is highly useful to studies of cellular biology as well as applications in diagnostics and therapeutics. However, currently available pH-sensitive nanoprobes suffer from photobleaching and autofluorescence background in biological samples. We designed a pH nanosensor that avoids these problems by taking advantage of near-infrared excitation with the use of upconversion nanoparticles (UCNPs) that emit visible light when excited with a 980 nm laser.

Oleate capped NaYF$_4$:Yb,Er nanoparticles were synthesized. A ligand exchange method was used to make the nanoparticles dispersible in aqueous solutions. Anthraquinones were then selected based on absorption bands that have strong overlap with the green emission of the UCNPs. This nanosensor relies on energy transfer between UCNPs and pH-dependent anthraquinone dyes for pH sensing.

We observed that upon NIR excitation, the green luminescence of the UCNPs is strongly absorbed by the anthraquinone dye. We have demonstrated pH response of these nanosensors in buffer solutions, and subsequent experiments are underway to explore pH-dependent enzymatic assays and pH measurements within the cell. Due to the advantages of upconversion photoluminescence, the nanosensor has the potential to be used for detection of pH in in vitro applications.

We would like to thank Junyang Huang and Will Deacon for assistance with the emission measurements, and Lisa Wiesholler, Markus Buchner, Sandy Himmelstoss, Verena Muhr, and Thomas Hirsch for assistance with the synthesis of UCNPs and for helpful discussions.

Keywords: Analysis, Biosensors, Nanotechnology, UV-VIS Absorbance/Luminescence

Application Code: Bioanalytical

Methodology Code: Sensors
In this paper we provide a new approach for modeling samples obtained from fluorescent microscope images; this work was motivated by ongoing Huntington's disease research. Huntington proteins (Htt) and green fluorescent proteins (GFP) were fused, inserted into yeast cells, and imaged using fluorescent microscopes in order to study the hypothesis that highly clustered proteins are less active and harmful to their host cells. The protein clusters appeared to change with changes in experimental parameters; however, investigators had no way to quantify these effects.

In our first attempt at quantifying this data, we extracted the boundaries of the bright regions (representing highly-clustered proteins) in the images and performed elastic shape analysis. This proved to be problematic because there was too much variation of intensities within a cell to provide meaningful analyses.

We overcame this problem by representing each cell as a three dimensional intensity surface. We identified a cell's boundary using Canny edge detection and separated touching cells using a watershed algorithm. We retained the pixel coordinates and used the pixel intensity values as coordinates. This resulted in a 3D surface representation where higher peaks correspond to higher levels of clustering. These surfaces became data inputs into an elastic surface modeling framework using a Square Root Normal Field mapping. Here, we calculated the average and variation of a sample of surfaces, and then mapped these surface statistics back to the original color space. Additionally, we performed two-sample hypothesis testing using the Fisher-Rao distance between densities estimated from the surfaces. The results show that representing samples obtained from fluorescent images as intensity surfaces and modeling them under an elastic framework provides a powerful new approach for providing statistical summaries and hypothesis testing for this previously intractable data.
Online Batch Correction and Interactive Data Visualization for GC/LC-MS Data

Batch effects on high-throughput GC/LC-MS dataset impede the finding of biomarkers and understanding of certain bio-process or environmental process. Such batch effects are hard to know before samples were analyzed and need correction. Currently, the correction methods of such unknown batch effects are not intuitively and hard to quantitate their influences. Meanwhile, current visualization methods of GC/LC-MS data were mostly based on targeted analysis and would bury important patterns in the data.

In this study, we employed simulation of batch effect based on the statistical properties of published datasets. Surrogate variable analysis (SVA) and Independent Surrogate variable analysis (ISVA) for unknown batch effects correction were applied to evaluate the batch correction. Based on the linear decomposition model of SVA, quantitative analysis and visualization methods are made to measure and show the influences from both batch effects and experimental design of each peaks. Such two-dimension description of influences could help to find robust bio-markers against unknown batch effects. The linear decomposition of raw data could also help to find the source of batch effects.

We also developed interactive data visualization method to make untargeted GC/LC-MS data intuitively. With new data visualization methods, one could filter the data and see the influences immediately. Such technique would benefit the data mining process during explore the data.

Both batch effect simulation/correction and interactive data visualization were integrated into a free open-source online application (https://yufreecas.shinyapps.io/xcmsplus/). Users could upload their own data in csv format to make batch correction and interactive data visualization. The R package supporting the application could also be found on CRAN (https://cran.r-project.org/web/packages/enviGCMS/index.html).

Keywords: Data Analysis, GC-MS, Liquid Chromatography/Mass Spectroscopy, Metabolomics
Application Code: Genomics, Proteomics and Other 'Omics
Methodology Code: Data Analysis and Manipulation
The high resolution mass spectrometer (HRMS) has been widely adopted by MS users. The HRMS data gives confident results and much more information, and HRMS usability has improved significantly over the years. However, HRAM data processing and interpretation has been a rate-limiting factor for many labs. Small molecule structure analysis is especially challenging due to its inherent complexity.

“Compound Discoverer 2.1” (CD 2.1) (Thermo ScientificTM) is a novel small molecule structure analysis software, the unique features and capabilities of CD 2.1 greatly increase the confidence and speed of small molecule structure ID. Through the node-based workflow, CD 2.1 utilizes accurate mass, isotopic pattern, and fragment ions to extract components and predict elemental compositions. Known compounds are identified through automatic online and offline multiple database and spectral library searching. mzCloud is a web-based, free searchable high resolution tandem MS spectral database. CD 2.1 uses query compound’s MS/MS fragment ions to search against mzCloud for known structure identification. It includes the mzVault™ application to support custom spectral libraries as well as a local version of mzCloud™ if MS systems are precluded from connecting to the Internet. Unknown components structure analysis is based on the predicted elemental composition and MS/MS fragmentation. In addition, the “mzCloud similarity search” function facilitates unknown compounds structure elucidation through identify the compounds from the database/library that are possibly similar or related to the query compound.

The unknown putative structures can be proposed in the “Compound Annotation Editor”. The “FISh Scoring” (Fragment Ion Search) function checks the proposed structure by searching the embedded “Fragments and Mechanism Library”. CD 2.1 generates scores for matching isotope, fragment, etc. They allow the user to evaluate the results rapidly and effectively.

**Abstract Text**

**Keywords:** Data Analysis, Data Mining, Liquid Chromatography/Mass Spectroscopy, Pharmaceutical
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**Abstract Text**

Drug registration authorities expect pharmaceutical development to demonstrate process and product understanding according to Quality by Design (QbD) principles with the overarching goal of ensuring medicines are safe & efficacious. To achieve those aims, control strategies need to be developed via approaches that involve assessing, classifying, reporting & ultimately controlling: process inputs and materials, their attributes, the design spaces around unit operations, methods, variability, and final product specifications. Impurity profiling data lies at the foundation of these approaches.

Software that facilitates the identification of process related impurities from well-managed and assembled analytical data and impurity fate mapping with chemical substance information related directly to the underlying data will be described. How helpful feedback of users enhanced the software will also be indicated.

**Keywords:** Database, Liquid Chromatography/Mass Spectroscopy, Pharmaceutical, Scientific Data Management

**Application Code:** Pharmaceutical

**Methodology Code:** Data Analysis and Manipulation
Continuous manufacturing, which is being strongly promoted by the FDA, is an innovative approach to pharmaceutical processing that utilizes information from multiple sensors throughout the entire process. Due to the numerous factors that need to be monitored and controlled at the same time, a multi-step approach is necessary for optimizing individual unit operations as well as the overall process. In this presentation we will show some approaches to combining process and spectroscopic data, which can be applied to process monitoring for both continuous and batch manufacturing.
Lewis and Edwards make the valid point that effort needs to be put into validating the accuracy and reliability of low cost air pollution networks, particularly those using electrochemical gas sensors. Importantly, network builders must also offer comparable data sets.

A major problem is the sensor zero-current response to changes in atmospheric humidity and temperature. Experimentally it is found that relative humidity transients cause impulse/decay changes in the zero current on the working electrode and these can take minutes to hours to return to equilibrium and are suggestive of changes in the sensor double layer.

We outline experimental techniques for identifying the system state response to changes in relative humidity and temperature. Statespace techniques are used to model the dynamics and compensate for zero current changes. Results are encouraging and open the possibility of generating meaningful confidence intervals.

Guidance is given to instrument builders on the constraints that must be observed in measurement technique in order to avail of the statespace compensation, particularly avoiding spectral distortion. To ensure that system builders can validate acquired datasets, a sensor manufacturer-accredited Software as a Service (SaaS) portal state compensation to system builders is described.

Ongoing work on data assimilation of distributed units into a system wide data confidence model is outlined. Nature Vol 535, Issue 7610, 06 July 2016 Validate personal air-pollution sensors
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**Abstract Text**

The scientific community has an ever growing need for good data management with security standards and streamlined information technology management. We have conducted a technology review of platforms that satisfy NIST requirements for data management, security, and information management. We discuss IAAS, PAAS, and SAAS cloud/hosted technologies as tools for meeting compliance requirements for the chemical and life science communities. We implemented a Microsoft based platform in Office 365 to determine the convenience, reliability, time efficiency and cost benefits of the platform for data analytics, regulatory reporting, and data management. We take case studies in various organizations and universities and present their experiences with the technology, platform and software. In addition, we demonstrate the environment reporting, tracking, file sharing, ediscovering, auditing, and collaborative and data recovery features.

**Keywords:** Data Analysis, Database, Scientific Data Management, Software

**Application Code:** Other

**Methodology Code:** Data Analysis and Manipulation
Herpes Simplex Virus (HSV) of Herpesviridae family is a double stranded DNA virus that is replicated and transcribed within host cell nucleus. It is predominantly responsible for oral and genital ulcer along with blister which is very much painful for infected individuals. It also potentially facilitates other sexually transmitted diseases (STD) like human immunodeficiency virus (HIV) infections. The multifaceted VP16 protein of HSV possess transcriptional regulatory domain which is highly active during viral infection. The 490 amino acids residue synthesized late during infection behave either as viral protein or cellular protein. The structured 16 VP.pdb 3D coordinate file of VP16 protein has been taken under screening different antiviral compounds for knowing presence of atomic level ligand protein interaction so that novel antiviral compounds against the HSV infection can be predicted. The drug discovery program called ligandfit of Discovery Studio v3.5. has been extensively used to detect presence of inhibitory action at atomic level of different antiviral compounds such as aciclovir, brivudine, entecavir, penciclovir, and tenofovir by docking method with VP16 protein of HSV. Among these compounds the LibDock Score of Aciclovir (102.004) is the highest one followed by Penciclovir (95.841). Both compounds have been used against HSV infection. But Entecavir (97.0294), Tenofovir (95.5972) and Brivudine (92.6344) are the good alternatives which are used in other viral infection treatment. The in vitro action of these antivirals is to be tested along with validating lessening of toxicity in pregnant women and children by following nanotechnology method such as PLGA-PEG conjugating with the antibody to the cells in which HSV infection occurs.
Carbohydrates play an important role in a variety of biological processes such as cell signaling, migration and cancer cell metastasis. Neutral and low molecular weight monosaccharides have recently become an alternative surface modifier for bioconjugation of plasmonic nanoparticles to the widely used bulky long chained poly(ethylene glycol) (PEG) due to their low cytotoxicity, stability and active targeting ability. In this study, we achieved the biofunctionalization of 4-aminophenyl D-glucopyranoside (Glu) and/or 4-aminophenyl D-galactopyranoside (Gal) onto 40 nm plasmonic gold nanospheres (AuNSs) through EDC/NHS chemistry. The nanometer precision non-fluorescent single particle tracking (SPT) of the monosaccharides modified AuNSs on both synthetic lipid membranes and live cell membranes using correlation mapping algorithm in differential interference contrast (DIC) microscopy was also demonstrated. Although the subtle difference on the monosaccharide structures result in similar lateral diffusion of the monosaccharides modified AuNSs on membranes, a dramatic difference on the internalization kinetics and efficiency was observed in live cell. Cholesterol level modulations elucidate the effects upon the changes of the cholesterol content in the membranes on the cellular uptake of the monosaccharides modified AuNSs, thus lead to the better understanding of the delivery efficiency of nanomedicines.
Anisotropic nanoparticles represent the state-of-the-art substrates for surface-enhanced Raman spectroscopy (SERS), and there is great interest in the development of facile synthetic strategies that achieve these morphologies in a seedless, one-pot, and surfactant-free manner. However, the absence of surfactants as shape directors poses challenges in maintaining the stability of the colloid, and detergents such as CTAB are generally added post-synthesis as stabilizers. This hinders the SERS signal via the formation of a bilayer that prevents a close contact between analyte and metal surface. Ideal capping agents for SERS substrates, on the other hand, should ensure a shelf-life to the colloid without interfering with the SERS measurement.

A published protocol for the surfactant-free synthesis of uncapped bimetallic Au/Ag nanostars was selected as a basis to study alternatives to CTAB as stabilizing agent to be added post-synthesis.

DLVO theory of colloid stability was used as a criterion for the selection of candidate stabilizing agents, and UV/Vis spectroscopy was used to evaluate the stability of the colloidal preparations over time. XPS, Zeta potential measurements, and infrared spectroscopy were used to investigate the surface chemistry of the nanostars, and the interaction modes of the candidate stabilizers. Preliminary results obtained from decay studies indicate a pattern of increased capping efficacy for candidate agents bearing carboxylate functions, suggesting that the driving force in the capping process is not purely electrostatic in nature.

Keywords: Molecular Spectroscopy, Nanotechnology, Surface Analysis, Surface Enhanced Raman Spectroscopy

Application Code: Nanotechnology

Methodology Code: Surface Analysis/Imaging
Herein, by utilizing localized plasmon resonance (LSPR) properties of metal nanostructures that result from the collective oscillation of their conduction electrons upon light irradiation we investigated reversible charge delocalization at the nanostructure-organic ligand interface. It is well known that the LSPR property is responsive to the concentration, dielectric thickness or refractive index of the attached self-assemble monolayer of ligands onto the nanostructure. Interestingly, our combined LSPR and surface-enhanced Raman Scattering measurements suggest that functionalization of gold nanostructure with para substituted conjugated thiols induces delocalization of conduction electrons wave function from nanostructure to ligand monolayer. Furthermore, our density functional theory calculations suggest that the appropriate alignment between the Fermi energy of nanostructure and the highest occupied (HOMO) and lowest unoccupied (LUMO) molecular orbitals energies of ligands are extremely important for efficient charge delocalization that resulted in previously unknown plasmonic properties of hybrid inorganic-organic nanomaterials. We believe this investigation will open new fields of scientific research and promote design of advanced biosensors, plasmon-enhanced photocatalysts, and metamaterials.
Cancer immunotherapy normalizes the immune system to harness immune system to treat cancer. Despite tremendous potential of molecular subunit vaccines for tumor immunotherapy, its clinic outcome has been suboptimal, largely due to inefficient co-delivery of heterogeneous peptide antigens and adjuvants to secondary lymphoid organs such as lymph nodes (LNs). Here, by conjugating albumin-binding Evans blue (EB) with vaccine (immunostimulatory DNA CpG adjuvant, B16F10 tumor antigen Trp2, and MC38 tumor-specific neoantigen Adpgk), we developed albumin-binding vaccine (AlbiVax) (Fig. a) that were co-delivered to LNs more efficiently (20-fold for CpG, 91-fold for antigen) than unconjugated vaccine or vaccine emulsified in Incomplete Freud’s Adjuvant (IFA) (Fig. b, f). Multi-scale imaging was employed to optimize and elucidate AlbiVax: first, by PET imaging of radiolabeled AlbiVax in small animals, we systematically optimized AlbiVax for optimal LN-targeted delivery (Fig. b, f); second, by light-sheet fluorescence imaging, we for the first time elucidated the distribution of AlbiVax in draining LNs that were cleared to be transparent (Fig. c, d); and third, by super-resolution imaging, we discovered efficient intracellular co-delivery of AlbiVax via albumin into endolysosome of antigen-presenting cells (APCs) (Fig. e). AlbiVax elicited 21-fold higher frequency of Antigen-specific CD8+ cytotoxic T lymphocytes (CTLs) than IFA-emulsified vaccine, and induced immune memory for > 5 months (Fig. g), making AlbiVax a potent T cell vaccine for cancer immunotherapy. AlbiVax dramatically regressed or inhibited the progression of established primary or lung metastatic EG7.OVA (Fig. g), B16F10 (Fig. h), and MC38 tumors (Fig. i). Combining AlbiVax with immune checkpoint inhibitor anti-PD-1 further improved the therapeutic efficacy and regression for MC38 tumor (Fig. h, i). Together, AlbiVax represents a widely applicable and robust T cell vaccine for cancer immunotherapy.

Keywords: Bioanalytical, Biopharmaceutical, Imaging, Nanotechnology
Application Code: Nanotechnology
Methodology Code: Microscopy
Nanomaterial-based field-effect transistor (FET) sensors are capable of label-free real-time chemical and biological detection with high sensitivity and spatial resolution, although direct measurements in high ionic strength physiological solutions remain challenging due to the charge screening effect. Here, we present a general strategy to overcome this challenge for FET sensors, which involves incorporating a porous and biomolecule-permeable polymer layer on the surface of FET sensors. We have used both silicon nanowire-based and graphene-based FETs, with polyethylene glycol (PEG) modification, for prostate-specific antigen (PSA) detection. Without the specific receptors, the FET devices exhibit clear and real-time reversible detection of PSA in 100 mM phosphate buffer, where the Debye length is similar to physiological conditions. In contrast, similar devices without PEG modification yield detectable signals only in low ionic strength solutions. Furthermore, studies carried out using PEG/DNA PSA aptamer-modified graphene devices show irreversible specific binding and detection of PSA in pH 7.4 1X phosphate buffered saline (PBS) solution, whereas control experiments with proteins that do not bind specifically to the aptamer show smaller reversible signals. In addition, the aptamer receptor of the modified graphene devices can be regenerated to yield multi-use selective PSA sensing under physiological conditions. We believe this work represents a critical step toward general application of nanomaterial-based FET sensors in many areas, including in-vitro and in-vivo real-time chip-based monitoring of disease marker proteins, which could have substantial impact on fundamental research and healthcare applications, as well as integration in freestanding nanoelectronic scaffolds for engineered tissues and in vivo implants.


**Keywords:** Bioanalytical, Biosensors, Nanotechnology, Protein

**Application Code:** Nanotechnology

**Methodology Code:** Sensors
Fluorescence spectroscopy has been used in many analytical applications in chemistry, biology, biochemistry and forensic analyses just to mention a few areas. One recent trend is the use of nanoparticles in these applications. Many different types of nanoparticles have been utilized for encapsulation of fluorophores, however silica nanoparticles have significant advantages in many applications. During the synthesis of silica nanoparticles it is relatively straightforward to introduce covalently copolymerized fluorescent dyes by using modified TEOS reactive analogues that are widely available. Also adding outside layers on to silica nanoparticle surface, chemical or sensor functionality can be introduced. For example we can change the hydrophobicity or pH sensitivity of the silica nanoparticle surface this way. During these studies surface properties of fluorescence silica nanoparticles were modified by adding hydrophobic or hydrophilic molecules on the surface to achieve biocompatibility. Biocompatibility was evaluated by hemolytic experiments. An additional application of hydrophobicity controlled fluorescent silica nanoparticles is the detection of latent fingerprints. The utility of this approach will be illustrated with high quality latent fingerprint images. Additional applications include silica nanoparticles containing several dye molecules as bright labels in immunochemical uses, cell imaging and forensic applications for latent blood detection. Surface bound moiety examples will be given for capillary electrochromatography using amino acid-bonded silica nanoparticles as pseudostationary phases as chiral selectors. Amino acid surface modified silica nanoparticles were used to achieve chiral separation of different enantiomers.

Keywords: Bioanalytical, Capillary Electrophoresis, Fluorescence, Forensics
Application Code: Nanotechnology
Methodology Code: Fluorescence/Luminescence
microRNAs (miRs) are small noncoding RNAs that are implicated in enhancing metastasis in breast, pancreatic, liver, and bladder cancers (BCs). State-of-the art PCR-based miR quantification requires biological fluid treatment, RNA extraction, labeling, amplification, and large samples, in addition to having measurement bias and variability, which together restrict its use in clinical point-of-care diagnosis. To overcome these challenges, we develop an ultrasensitive, nanoparticle-based, multiplexing sensor utilizing gold nanoparticle localized surface plasmon resonance (LSPR) to optically assay miRs in plasma with unprecedented selectivity without labeling and amplification. Our technique is capable of detecting 15 different miRs simultaneously (including the false negative false positive) with femtomolar sensitivity in plasma of cancer patients sample without any prior extraction or purification. The demonstrated sensitivity and feasibility of our novel methodology, with potential ability to establish high-throughput multiplex assays, will allow rational design of a stable, low cost medical device as a realistic alternative to qRT-PCR for testing the utility of miRs as cancer markers for early disease diagnosis.
Nanostructured Polyamic Acid Membranes for Anti-Microbially Enhanced Water Treatment Cartilages

Water scarcity and quality challenges facing the world can be greatly alleviated by Point-of-Use filtration devices (POU). The use of filtration membranes in POU devices has been limited largely because of membrane fouling. Membrane fouling occurs when suspended solids, microbes and organic materials are deposited on the surface of filtration membranes and significantly decreases the membrane lifespan and increases operation costs. There is need therefore to develop filtration membranes that are devoid of the challenges posed by foulants and that can actively participate in the filtration process. Nanotechnology shows great potential to alleviate this challenge. Silver nanoparticles can be cheaply synthesized and have for the longest time been known as impressive anti-microbial agents. Gold has as well attracted great research interest owing to its fascinating catalytic activities demonstrated in assorted scientific research works. The objective of this work was to prepare and characterize hydrophilic Poly (Amic acid) polymer films containing Silver and Gold nanoparticles and evaluate their suitability as antimicrobial materials for water treatment. Hydrophilicity of PAA is a deterrent to foulant adhesion to the membrane’s surface while gold NPs catalyses the interactions of silver NPs with microbes for better antimicrobial properties. We will report the synthesis and fabrication of Nanostructured Polyamic memberanes and their application in water filtration. The nanostructured thin films were fabricated via thermal and wet phase inversion techniques and then tested against the microbes Escherichia coli and Staphylococcus aureus following standard tests.

Keywords: Adsorption, Analysis, Environmental/Waste/Sludge, Membrane

Application Code: Nanotechnology

Methodology Code: Process Analytical Techniques
Sample Preparation and Extraction

Modelling and Experimental Evaluation of the Sampling and Post-Sampling Processes in a Permeation Passive Sampler

Abstract Text
A mathematical model was developed and numerically evaluated to describe the sampling process in permeation passive sampling. The model was applied to a polydimethylsiloxane (PDMS)-based permeation passive sampler and evaluated using two types of sorbent with different properties and sorption strength. The model results demonstrated changes in the uptake rates of the sampler with sampling time at rates that depend on the concentration level and the sorbent-analyte combination. The model allows better understanding of the sampling process and of the factors affecting the accuracy of the time-weighted average (TWA) concentration measurement using passive samplers. This understanding permits guided optimization of the sampler design, sorbent material, and sampling time for the desired sampling purposes. A method for correcting the calculated concentration based on the effective uptake rate predicted by the model for a given exposure was also developed and evaluated. The work also evaluated the distribution of analytes within different components of the sampler at the end of the sampling time, the changes in this distribution during the post-sampling period, and the efficiency of the current storage method both theoretically and experimentally. The model results and their experimental validation will be presented.

Keywords: Environmental Analysis, Monitoring, Sampling, Volatile Organic Compounds
Application Code: Environmental
Methodology Code: Sampling and Sample Preparation
Sample preparation by fusion is known as being the best technique to enhance accuracy and precision of XRF analytical results for a wide range of samples. However, special considerations have to be taken into account when preparing the sample prior to the fusion step in order to benefit from all the advantages of this preparation technique. One of the most decisive factors is the flux-to-sample ratio. It has an impact on the speed and degree of chemical reaction during the fusion process, the final mass absorption coefficient of the measured sample and the dilution factor to be applied to the analysis. The flux-to-sample ratio is directly affected by weighing errors that arise from the lack of precision of the weighing technique.

This study compares the most common weighing techniques used in preparing samples for fusion: catch weight and sample-to-flux ratio. Different weighing precisions have been tested. Manual and automated weighing as well as XRF correction have been compared. This study shows that even when using high-performance fusion equipment and an adequately calibrated spectrometer, small weighing errors and mediocre weighing methods lead to poor overall analytical reproducibility. We also demonstrate that automated weighing saves significant time and reduces operation costs.

**Keywords:** Elemental Analysis, Instrumentation, Sample Preparation, X-ray Fluorescence

**Application Code:** Other

**Methodology Code:** Sampling and Sample Preparation
Solid phase microextraction (SPME) is a solvent-free sample preparation technique that uses fibers coated with various solid and liquid extraction phases. Recently we have developed a new generation of SPME materials that are highly porous, deposited by sputtering. The resulting silicon/silica coatings can be silanized, e.g., with a C18 silane ([i]Anal. Chem.[/i], [b]2016[/b], [i]88[/i] (3), 1593–1600). In this presentation, it will be described different ways in which the porosity and thicknesses of the silicon coating can be controlled, and it will also be described about new stationary phases on these coatings. These new fibers/coatings and/or model/witness substrates that represent them have been characterized by spectroscopic ellipsometry, water contact angle goniometry, and X-ray photoelectron spectroscopy. The performance of the resulting SPME fibers in both headspace and direction immersion modes is demonstrated. Various specific applications will also be discussed, e.g., the analysis of polyaromatic hydrocarbons. These fibers showed better stability, higher capacity, more reproducibility and robustness in comparison to the commercially available fibers.

Keywords: GC-MS, PAH, SPME, Water
Application Code: Environmental
Methodology Code: Sampling and Sample Preparation
Sample Preparation and Extraction

Supercritical (SFE)/Subcritical (subFE) Fluid Extractions for Drug Product Analysis: Rapid Assessment of Tablet/Capsule Potency

Tablet potency is a standard critical quality attribute for finished drug product that ensures clinical efficacy, patient safety, and product performance. Currently, potency is evaluated using a series of tedious sample preparation steps and analysis by liquid chromatography (LC). Sample preparation (both manual and automated) involves time consuming, solvent intensive disintegration methods where sonication and/or mechanical stirring is used to free the active pharmaceutical ingredient (API) from the tablet matrix. These sample preparation steps can take up to 80% of the total analysis time. In addition, resource intensive method development timelines are significantly reduced by application of a general, time efficient extraction method. In this work we present simple sample preparation workflows to rapidly develop tablet potency assays using supercritical (SFE)/subcritical (subFE)-based CO2 extractions. With preliminary solubility data on the API, this method screening workflow evaluates extraction efficiency for each API as a function of SFE parameters, i.e. temperature, pressure, flow rate, modifier, etc. (Extraction efficiency is evaluated using offline LC). Of particular importance to extraction efficiency is extraction fluid composition. Water is used to facilitate tablet disintegration, with secondary polar organic modifiers dramatically improving extraction efficiency of polar, highly potent APIs. To date we have applied this proof-of-concept procedure to a series of commercial pain relievers (e.g. acetaminophen and ibuprofen), antihistamines (diphenhydramine, cetirizine and loratadine) and commercial Lilly products.

Abstract Text

Keywords: Extraction, Pharmaceutical, Sample Preparation, SFE
Application Code: Pharmaceutical
Methodology Code: Sampling and Sample Preparation
Breath is an important biological medium for clinical evaluation of human health and determination of environmental exposures. In addition to the gas-phase of exhaled air, exhaled breath aerosols (EBA) and exhaled breath condensate (EBC) are breath media that provide additional information about an individual’s health state. The development of non-invasive sampling methods for collection of breath media is desired to remove the need for medical personnel and to allow for more flexible sample volumes and frequency. Furthermore, individuals are more likely to participate in a study if sampling is quick and painless. We have developed sampling protocols for EBA collection using filter swipes of different types of masks worn by human volunteers. Volunteers wore the masks and breathed normally for at least 10 min prior to sampling. The surfaces of the masks where exhaled breath particles may have come in contact with were sampled using moistened filter swipes. The dried filters were extracted using two different protocols for proteins and condensed phase molecular analyses. Cytokine levels were assessed using two immunoassay instruments with different sensitivities for comparison between a variety of breath samples from EBA and EBC. Sample extracts were also subjected to LC-QTOF mass spectrometry to identify compounds produced by EBA. Cytokines, fatty acids, and a variety of other biological compounds were identified in the breath samples. This research was conducted with IRB approval for human subjects and biological specimens.
Sample Preparation and Extraction

Innovative Cryogen-Free Ambient Air Monitoring in Compliance with US EPA Method TO-15 and Chinese EPA Method HJ 759

Monitoring of chemicals in ambient air is necessary to determine the effect they have on human health, the environment and global climate. This has driven a number of national and international regulations to be developed, primarily in response to increased concern over potentially hazardous volatile organic compounds (VOCs) in ambient (primarily urban) air, industrial emissions and landfill gas. Analysis of these VOCs is carried out in accordance with a number of standard methods, which require the use of either sorbent tubes (pumped or passive), canisters, or on-line techniques. Each method has its own advantages and range of applicability, with canister sampling being most popular in the US and China. To achieve the required detection limits using this approach, pre-concentration is required to focus analytes and selectively eliminate bulk constituents, and this approach is mandated within the most popular standard method for canisters, US EPA Method TO-15. China also released a similar methodology in 2015, namely Chinese EPA Method HJ 759, which defines the sampling and analysis of VOCs in ambient air using canisters. This paper describes the GC–MS analysis of humidified canister ‘air toxics’ samples at various relative humidities, using cryogen-free systems for thermal desorption preconcentration. Detection of 65 target compounds ranging in volatility from propene to naphthalene is demonstrated with excellent peak shape and performance well within the criteria set out in US EPA Method TO-15 and Chinese EPA Method HJ 759, including method detection limits as low as 4 pptv.

Abstract Text

Keywords: Air, Capillary GC, Environmental/Air, GC-MS
Application Code: Environmental
Methodology Code: Sampling and Sample Preparation
Sample Preparation and Extraction

Development of Custom, Microporous, Hydrophilic Lipophilic Balanced (HLB) Particles for the Preparation of Balanced Coverage Thin Film Solid Phase Microextraction (TF-SPME) Devices

Solid phase microextraction (SPME) devices, used for gas chromatography based determinations use DVB-PDMS and Carboxen sorbents. These materials target non-polar volatile and semi-volatile organic compounds by hydrophobic interactions. However, these are little desired in terms of polar analytes coverage. To compensate these limitations, thin film-SPME devices which exhibit surface area were devised. Even still, most work in the field of TF-SPME has continued to use hydrophobic sorbents. Mesoporous HLB sorbents have been used to address this limitation. Henceforth, this work presents in-house development and comparison of a microporous HLB particle based TF-SPME device for balanced coverage. The proposed, HLB particles were 1.33 µm in size, prepared by precipitation polymerization and tailored to be microporous in nature for GC applications, exhibiting pore diameter of 13 Å which is smaller than the 80 Å pore present in commercially available HLB. Despite, smaller pore diameter and particle size, the chosen porogen gave HLB particles having a specific surface area of 335 m2/g, about half of commercial particles. For reference another HLB sorbent was prepared in house using a suspension polymerization having characteristics similar to the commercial particles, TF-SPME membranes were prepared using the 3 HLB chemistries described and compared to traditional DVB/PDMS chemistry. In comparison to blank desorption the HLB/PDMS membranes show no more background bleed than the DVB/PDMS analogue. No carryover of the modified McReynolds standard could be detected on the HLB/PDMS membranes. These HLB/PDMS TF-SPME membranes were found to extract a factor of 1.3x-2.5x more for benzene, 2-pentanone, 1-nitropropane, pyridine, 1-pentanol, and octane than the established DVB/PDMS based membrane. Furthermore, membranes prepared using these house made particles has shown to extract a significantly ($p= 0.00047$) greater amount of analytes than membranes having commercial HLB particles.

Keywords: Sample Preparation, Sampling, SPME, Thermal Desorption
Application Code: Material Science
Methodology Code: Sampling and Sample Preparation
Sample Preparation and Extraction

Evaluation of Reproducibility and Matrix Compatibility of SPME LC Tip Fibers for Compounds with A Wide Range of Physicochemical Properties

The development of new biocompatible SPME phases capable of covering a wide range of polarities is garnering a wealth of interest in the bioanalytical field. The purpose of this work is to present a new generation of SPME coatings based on mixed-mode mechanisms of extraction. Essentially, the sorbent used consists of a combination of a C18 moiety that enables different Van der Waals interactions, and a benzene sulphonic acid group that allows for strong cation exchanging properties. This coating chemistry was evaluated by employing a group of model compounds covering a wide range of physicochemical properties. Individual coating batches were characterized in terms of extraction performance, inter-fiber reproducibility, reusability, coating stability, and batch reproducibility. For the evaluation of this new generation of mixed-mode fibres, consecutive extractions were conducted in various matrices including phosphate buffered saline solution (PBS), blood, plasma, urine, and grape juice. The evaluated fibers in PBS extractions showed an average inter-fiber reproducibility ranging from 5.6% to 22% depending on the model compound and extraction time. Interestingly, the most polar analyte selected for this study (morphine, logP: 0.86) could not be detected in any of the studied matrices at a concentration level of 50 ng mL-1. The rest of model analytes showed an increasing affinity towards the tested coating material as their polarity decreased. In regard to the matrix compatibility, the evaluated fibres exhibited rewarding results in terms analytical precision corresponding to extractions in various biofluids (%RSD from 0.4% to 9.5%). Moreover, such coatings did not show significant morphological changes after three consecutive extractions in different biological matrixes. Differences in recoveries obtained in the evaluated matrixes were related to the affinity binding constants of each compound for the correspondent matrix, and not to coating matrix effects.

Keywords: Bioanalytical, Biomedical, Liquid Chromatography/Mass Spectroscopy, SPME
Application Code: Bioanalytical
Methodology Code: Sampling and Sample Preparation
N',N''-(6-(4-phenylpiperazin-1-yl)-1,3,5-triazine-2,4-diyl)dibenzohydrazide (3a-f) were synthesized by reaction of 2,4-dichloro-6-(4-phenylpiperazin-1-yl)-1,3,5-triazine and various benzohydrazide derivatives. All the 3a-f derivatives were characterized by elemental analysis and IR spectral studies. All the 3a-f compounds were evaluated for antimicrobial activity against different microorganisms.

All these derivatives were employed as epoxy resin curing agent. Thus the curing of commercial epoxy resin namely diglycidyl ether of bisphenol-F (DGEBF) was monitored on Differential Scanning Calorimeter (DSC) and Thermo gravimetric analysis (TGA). The glass-fibre reinforced composites (i.e. laminates) based on DGEBF-(3a-f) systems were prepared and characterized by physical and mechanical data.

Keywords: Characterization, DSC, Elemental Analysis, FTIR

Application Code: Material Science

Methodology Code: Atomic Spectroscopy/Elemental Analysis
Free moisture and loss on ignition (LOI) of Hydrated Lime, Limestone, and Cement measurement is utilized for determining the mass of material that will be lost when fired in a kiln. The analysis of the material is also a metric for the purity of the raw and processed material. The determination of LOI using a LECO TGA701 macro analyzer may be separated into distinct temperature steps to distinguish between mass loss from hydroxyl groups and the release of CO2 or SO2. Current methods for free moisture and LOI of Hydrated Lime are performed in a muffle furnace according to ASTM C25, while analysis for Cements is performed according to ASTM C114.

Macro TGA instruments typically utilize a gram-sized sample with a high temperature furnace using an air or oxygen atmosphere for moisture and LOI determination. The samples are weighed into ceramic crucibles located on a rotating carousel, utilizing a high precision internal balance (0.0001g). The sample batch undergoes a pre-programmed analysis method with several steps to determine mass loss due to free moisture and LOI. During the analysis, the samples are continuously weighed in sequence to monitor the mass loss at each step without the removal of the sample from the furnace.

This poster presentation will cover the determination of free moisture and LOI of hydrated lime, limestone, and cement utilizing a LECO TGA701 Macro analyzer with results presented and data evaluated.

Keywords: Environmental, Geochemistry, Materials Characterization, Thermal Analysis

Application Code: Material Science

Methodology Code: Thermal Analysis
The discharge of volatile matter from the interiors of automotive materials is one of the most direct reasons for air quality and causing diseases for vulnerable people. The main purpose of this research is to establish a simple and quick method to analyze the odorous compounds in interiors of new cars, which could provide imperative information for vehicle decoration manufacturing. A GC-MS/MS odor database including parameters, sensory information and other data for about 150 of the primary compounds that cause odors was used, while the detected compounds retention times can be precisely calculated by retention indices which are already registered in the odor database. Standard mixture sample of 4-bromofluorobenzene, 1,2-dichlorobenzene-d4, and acenaphthene-d10 were used for calibration curve correction, so that semi-quantitative values can be calculated for detected components. Headspace solid-phase microextraction (HS-SPME) was recommended to enhance the efficiency of extraction of odorous compounds. Samples offered by a car manufacturer were divided into two groups. The first group contained normal seat leather and foam with an unpleasant odor, in contrast, the other group had the seat leather and foam special treated without peculiar smell. The results showed that 14 odorous compounds were detected in both seat leather and foam. 1-Octanol, Acetophenone, 2-Ethyl-1-hexanol, 1-Dodecanol, Naphthalene, 2-Methylnaphthalene, DBT, etc. made a great contribution to the unpleasant smell of seat leather and foam according to the odor threshold values (odor threshold is a numerical value of the minimum concentration of the odorous compound which people can react) registered in the odor database. Even though some odorous compounds were detected in the special treated group, on the contrary, the concentration of the odorous compounds in the special treated were lower than the odor threshold.
With the continual decrease in device geometries, the trace element content of process chemicals, as defined by Semiconductor Equipment and Materials International (SEMI), are moving to increasingly lower levels. Mineral acids are employed in various stages of device manufacture; for example H2SO4 is used in the etching process of wafer production and high levels of trace element impurities will decrease the yield of the produced IC (integrated circuit) devices. Current or proposed SEMI guidelines for many mineral acids require sub-ppb concentration maximums in the undiluted mineral acid. Techniques (e.g. evaporation etc.) to remove the troublesome mineral acid matrix are no longer favored due to the increased sample handling and risk of trace element contamination. The use of different ICP-MS technologies using fully automated sample handling systems for the simple, direct analysis of process chemicals used in the semiconductor industry (e.g. HNO3, H2SO4, etc) will be shown. In order to address matrix induced spectral interferences, the combination of hot and cold plasma with flexible collision cell methodologies, Triple Quadrupole ICP-MS or High Resolution ICP-MS will be demonstrated. Another typical semiconductor analysis is vapor phase deposition on the silicon wafer with ICP-MS detection (VPD-ICP-MS). VPD has limited sample volume and high acid concentration. Automated sample handling that circumvents the handling of hydrofluoric acid by the operator and improves lab safety and efficiency will also be presented.
3,3’-(4,4’-(6-morpholino-1,3,5-triazine-2,4-diyl)bis(azanediyl)bis(4,1-phenylene)) diacylic acid (MTAPA) was prepared by reaction of 4-(4,6-dichloro-1,3,5-triazin-2-yl) morpholine and 4-Amino Cinnamic acid derivative was characterized by elemental analysis, acid value and spectral studies.

MTAPA 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 commercial USPE 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.

Keywords: Characterization, DSC, Elemental Analysis, FTIR
Application Code: Material Science
Methodology Code: Atomic Spectroscopy/Elemental Analysis
A series of new heteroaryl azo dyes were synthesized by coupling of diazonium salts of various phenyl amine with newly synthesized imidazole - thiazole combined compounds. N-((1H-benzo[d]imidazol-2-yl)methyl)-4-(4-chloro phenyl)thiazol-2-amine. It was prepared by simple condensation reaction of 2-(chloromethyl-1H-benzo[d]imidazole with 4-(4-chlorophenyl) thiazole-2-amine derivative. It was characterized duly various benzenesulfonic acid were diazotized and the resultant diazonium salts were coupled with above compound. The so-called result dyes 4-((2-(((1H-benzo[d]imidazol-2-yl)methyl)amino)-4-(4-chlorophenyl) thiazol-5-yl)diazeny)benzenesulfonic acid (Dye-A) obtained were characterized by elemental content, acid azo group determination and spectral studies. The dyeing performance of these dyes where assessed on wool and nylon fabric. The dyes patterns were of various shades of red and orange with good depth brightness and leveling properties. The dyed fabric showed fairly good to very good light fastness and very good to excellent fastness to washing, perspiration and rubbing. The dye bath exhaustion and fixation on the polyester fabric was found to be very good.

Keywords: Characterization, FTIR, Spectroscopy, Temperature
Application Code: Material Science
Methodology Code: Chemical Methods
A cathode material, Li$_3$V$_2$(BO$_3$)$_3$, has been prepared with citric acid by sol-gel route at various temperatures ranging from 400 to 600°C and characterized for optimization of calcination temperature. The electrochemical performances of Li$_3$V$_2$(BO$_3$)$_3$ as a cathode material for LIBs was investigated. Citric acid acts not only as a chelating agent but also as a carbon source, which enhance the conductivity of the composite material and hinder the growth of Li$_3$V$_2$(BO$_3$)$_3$ particles during preparation. Also, the structure and morphology of Li$_3$V$_2$(BO$_3$)$_3$ were characterized by X-ray diffractometry (XRD) and scanning electron microscopy (SEM). Galvano-static charge/discharge and cyclic voltammetry (CV) measurements were used to study its electrochemical behaviors which indicate the reversibility of the lithium extraction/insertion processes. The sample synthesized at 450°C exhibits the highest capacity of 198 mAh/g and excellent cycleability.
The scope of this work throw out the light on synthesis an engineered poly B-aminoester (PBAE) modified with polyvinyl pyrrolidone loaded curcamine as multi effective natural product as a trial system to release the active material (curcamine) to overcome some dangerous diseases like cancer and alzheimer. Poly B-aminoester was synthesized from specific ratios of 4,4/tetramethylenedipipredene and 1,4 butanedioldiacrylate in tetrahydrofuran at 50°C for 72 hrs and purified by excess of n-hexane and stored at 4°C. It was modified with different ratios of polyvinylepyrrolidone in absolute ethanol before the end of the reaction curcamine was added for holding. They were added to phosphate buffer saline PH 7.4 under ultrasonication. The suspension was centrifuged at 10,000 rpm for 20 min. at 4°C to obtain the investigated nanocomposite. They were characterized by FT-IR, TEM, SEM. It was found that the size of the nanocomposite particles was about 16-23nm. The release of curcamine in aqueous media was measured spectrophotometrically. The results indicated that about 44% of curcamine consumed after 7 days from the prepared aminoester before modification and 50% after modification. The efficiency of curcamine loaded on the investigated polymer was 97%. The efficiency of released curcamine on growth inhibition and survival of liver cancer cell line show high growth inhibition i.e. promising results. So, it is concluded that, curcamine as a safe natural material can be used as effective anti cancer drug specially when applied as sustained release technique from polymeric system. This technique was used to manipulate the application of drug to gain maximum efficiency and overcome the side effect.

**Keywords:** Drug Discovery, Identification, Natural Products, Spectrometer

**Application Code:** Material Science

**Methodology Code:** Chemical Methods
Creating a p-n heterojunction in metal oxide semiconductors has proven effective approach to decrease the electron hole recombination and enhance the photocatalytic activity. Pd/m-BiVO4/BiOBr ternary composite has demonstrated substantial photocatalytic activity in the degradation of organic contaminants. To understand the correlation between the rational composition of the p-type and n-type semiconductors in the heterojunction material and the photocatalytic activity, we controlled the molar ratio of ammonium vanadate relative to bismuth nitrate in the reaction. Changes in the morphology and composition of the material were characterized by scanning electron microscopy and energy dispersive spectroscopy. Pure BiOBr nanosheets were afforded from the reaction of bismuth nitrate and cetyltrimethylammonium bromide at 80 °C. The gradual increase of the amount of ammonium vanadate added into the reaction resulted in formation of heterojunction composite of m-BiVO4/BiOBr with steady decrease in the ratio of BiOBr in the composite. Diffuse reflectance spectroscopic analysis of the composites showed a band gap shifted from ~3.1 eV at 0 mol% vanadate to dual band gaps of ~2.4 and 3.1 eV starting at 30 mol%. An m-BiVO4 rich composite was achieved by increasing the ammonium vanadate to 100 mol% with single band gap of ~ 2.45 eV. The change in the composition of the m-BiVO4/BiOBr demonstrated significant influence on the photocatalytic degradation of rhodamine B. The amount of palladium nanoparticles was found to have direct relationship with the activity of the photocatalyst. These results allow for an intuitive understanding of the material’s structure-related photocatalytic activity of metal oxide semiconductors.
We have demonstrated the ability to induce a free charge density gradient over macroscopic distances (ca. 100 µm) in the room temperature ionic liquid (IL) 1-butyl-3-methylimidazolium tetrafluoroborate (BMIM+BF4-), supported on a charged surface. We have used fluorescence-lifetime imaging microscopy (FLIM) to measure the rotational diffusion dynamics of three structurally similar chromophores (anionic, cationic and neutral) as a function of distance from the charged support. These data exhibit rotational diffusion times that vary by as much as 25% as a function of distance from the support. The same measurements in ethylene glycol reveal no distance dependence. Using a conductive oxide support, FTO (fluorine doped tin oxide), allows explicit control over the bias applied to the support, and we report how the reorientation dynamics of the charged chromophores varies with the potential of the FTO support. These data demonstrate control over organization in an IL that exceeds previously reported gradients in this family of materials by as much as five orders of magnitude. There are many potential applications for this effect, including energy storage and dissipation, and control over electric field propagation.

**Keywords:** Analysis, Fluorescence, Laser, Microscopy

**Application Code:** Material Science

**Methodology Code:** Microscopy
A set of binary mixtures styrene/alcohol has been studied from two different points of view. As solvent polarity parameters, dielectric constants have been measured in the entire range of composition and transition energies ET(30) using the Reichardt’s betaine dye as probe. The following solvents pentanol, butanol, propanol, ethanol and methanol were used as cosolvents. Measurements were carried out at 20°C. Dielectric constants were measured with an oscillometer operating at 5MHz. Charge transfer spectra due to the probe dye, were recorded on a double beam UV-Vis spectrophotometer. Hydrogen bonded OH effects were detected on an FT infrared spectrometer provided with a HATR accessory. Plots of dielectric constants or ET(30) values against alcohol mole fractions are nonlinear, then the systems are nonideal. Negative deviations characterizes dielectric data and positive deviations in case of ET(30) values. All of these functions are polynomials of degree 3.

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Keywords: FTIR, Material Science, Solvent, UV-VIS Absorbance/Luminescence
Application Code: Material Science
Methodology Code: Molecular Spectroscopy
Analytical Applications in Material Science

Hierarchical Porous Carbon Nanomaterials Derived from Wasted Biomass as Oxygen Reduction Reaction Electrocatalysts

The oxygen reduction reaction (ORR) and oxygen evolution reaction (OER) are decisive steps in efficiency improvements of energy conversion storage techniques such as in metal–air batteries, fuel cells and water-splitting[1]. Although Pt-based catalyst has been currently recognized as the most significant competitor toward the state-of-the-art electrocatalysts for ORR, the high cost and scarcity, as well as poor durability, susceptibility to gas poisoning, and a negative environmental effect inevitably hindered its commercial application[2]. The design and synthesis of highly active and long-term durable carbon-based electrocatalysts to take the place of noble metal remains a big challenge. Herein, we utilize various waste biomass as raw materials to synthesize hierarchical porous carbon materials. The resultant material has a high BET surface area, abundant hierarchical porosity and shows the impressive electrocatalytic performance, durability and excellent methanol tolerance in pH-universal media for the oxygen reduction reaction (ORR) and alkaline media for the oxygen evolution reaction (OER). This strategy provides a brief pathway to prepare outstanding N-doped carbon-based electrocatalysts straight forward from waste biomass.

Co-Author(s)

Hong-Ying Zang
Northeast Normal University

Abstract Text

Keywords: Electrochemistry, Electrodes, Material Science
Application Code: Material Science
Methodology Code: Electrochemistry
Surface-enhanced Raman scattering (SERS) is an emerging analytical technique used for the identification and characterization of chemical and biological molecules or structures. The SERS enhancement is based on two mechanisms: electromagnetic enhancement and chemical enhancement. Since electromagnetic enhancement is the major contributing mechanism, research focuses on targeted engineering of novel plasmonic structures to obtain high enhancement factors while maintaining reproducibility across the substrates. Up to date, gold (Au) and silver (Ag) plasmonic nanostructures are most commonly used as SERS substrates due to their higher enhancement factors and availability of plasmonic resonances in the visible and NIR regions. Recently, there has been great attention on diatoms for their potential applications in next generation biomedical applications, such as drug delivery, biosensing, and membrane technology. There is also evidence showing that diatoms can be utilized in improving SERS enhancement by optically coupling guided-mode resonance (GMR) of the diatom frustules with the LSPRs of the nanostructures resulting in a lower limit of detection. In addition to this increase in enhancement, the large surface area of the porous diatom structure increases the chance of analyte attachment, which is significant for SERS-based biosensing. In this study, layer-by-layer assembly method is used to obtain metallic nanoparticles/diatom composites for SERS active platform. The obtained composites are characterized using scanning electron microscopy (SEM), Zetasizer and UV/Vis. Spectroscopy. SERS performance of the prepared composites is assessed using 4-aminothiophenol (4-ATP) and rhodamine 6G. The results demonstrate that, SERS performance is strongly dependent on the type of the metal nanoparticles and number of layers on the diatoms. The composite having highest enhancement factor can be for SERS-based identification and characterization of molecules and structures.

**Keywords:** Material Science, Surface Enhanced Raman Spectroscopy

**Application Code:** Material Science

**Methodology Code:** Vibrational Spectroscopy
Gallium Indium Eutectic Masking during Porous Silicon Formation Leads to Unique Analyte Photoluminescence Responses

Porous silicon (pSi) has been explored as a platform for multi-analyte detection. Here we show that one can use gallium indium (GaIn) eutectic to create interesting crystalline Si/porous silicon (cSi/pSi) platforms that exhibit unique analyte- and spatially-dependent photoluminescence (PL) responses. This presentation focuses on characterizing these cSi/pSi interfaces by using profilometry, scanning electron microscopy (SEM), wide-field multispectral PL microscopy, and Fourier transform infrared (FTIR) microscopy. As we move along a vector from the cSi/pSi interface out into “bulk” pSi, our results reveal that the: (i) analyte-dependent, PL-based response initially increases and then decreases; (ii) total PL emission intensity increases; (iii) pSi thickness increases; and (iv) relative O[sub]2[/sub]Si-H to Si-H band amplitude ratio decreases. The analyte PL response is therefore correlated to pSi oxidation extent. This presentation will summarize our most recent results and interpretations.

Keywords: FTIR, Luminescence, Material Science, Microscopy
Application Code: Material Science
Methodology Code: Fluorescence/Luminescence
Ionic liquids (ILs) are organic salts which possess appreciable liquid range, owing to their high thermal stabilities and low melting temperatures. Their tunable properties made them excellent candidate for plethora of applications. Within ionic liquids, dicationic ILs usually show higher thermal stability as compared to conventional monocationic ILs. Thanks to their larger molecular weight, and higher charge which results in greater interactions between dicationic moiety and paired anions, these salts have very low volatility even at high temperatures. In addition, dicationic ILs have higher viscosities than monocationic ILs. However, increased electrostatic interactions between charged species causes an increase in the melting points of dicationic ionic liquids. Herein, we report the synthesis and characterization of 77 dicationic ionic liquids. Structural variations were performed on cationic head groups, counteranions, and spacer chain. Imidazolium, pyrrolidinium, and phosphonium based ILs were paired with different anions containing either sulfonyl or sulfonate groups. In addition, the effect of alkyl substituent on positively charged groups as well as alkyl linker was investigated. The synthesized dicationic ILs were characterized in terms of their physical properties such as viscosity, liquid density, and melting point. A more comprehensive study was conducted on thermal stabilities of these ionic liquids as a function of time-dependent process, utilizing TGA and inverse gas chromatography.

We appreciatively acknowledge the Robert A. Welch Foundation (Y0026) for the support of this work.

Keywords: DSC, High Temperature, Material Science, Thermal Analysis
Application Code: Material Science
Methodology Code: Thermal Analysis
In this talk we will showcase a recent example where we carried out in situ monitoring of a complex network of reactions by combining the power of NMR, Raman, IR and NIR. Two reactions involving the hydrolysis and condensation of phenyltrimethoxysilane and methyltrimethoxysilane were used as examples. A wealth of information can be obtained by such a comprehensive analytical approach. For example, the sequential hydrolysis involving multiple intermediate products could be followed and quantified by NMR. The NMR results could in turn be used to calibrate the vibrational spectroscopy results. Such calibration was not otherwise possible as many of the intermediate species are highly unstable and cannot be prepared by mixing known standards together. The strengths and weakness of each technology will also be discussed.
A novel approach was utilized to study the influence of column bleed of stationary phases on signal suppression, adduct formation and sensitivity drop in detail. Two options were taken into account in order to minimize these negative effects:

- Implementation of a straightforward column washing process
- Development of new stationary phase materials

The result of both approaches was monitored using gradient HPLC-MS. Various HPLC-MS solvents as well as buffers were analyzed in terms of their purity utilizing flow injection analysis (FIA) or LC-MS on a standalone MS system. A FIA was further applied to analyze the pitfalls of solvent and additive handling and storage on sensitivity as well as on adduct formation and signal suppression of a model setup.

Keywords: HPLC, Mass Spectrometry, Quantitative, Solvent
Application Code: Bioanalytical
Methodology Code: Liquid Chromatography/Mass Spectrometry
Every year over 200,000 children are diagnosed with autism in the United States alone. Now, one in every 45 children between the ages of 3-17 have been diagnosed with the condition. With so many new cases each year, physicians need ever more sophisticated tools for early diagnosis and prevention. The lab has identified several potential clinical markers which may aid in this diagnosis. One such marker is methylmalonic acid concentrations in children on the spectrum which, in previous experiments, was determined to be higher in children with autism $p$-value = 0.0042 ($n$=30 pair of children, 95% CI, control cohort: 130 ± 14, autism cohort: 193 ± 40 (nmol/L)). Previous experiments involved analyzing blood serum by traditional calibration curve methodology through a commercial clinical lab using phlebotomy. Moving forward the lab is developing new methodology for the quantitative analysis of methylmalonic acid; comparing the traditional calibration curve method to our labs’ patented IDMS method (based on our EPA Method 6800). IDMS eliminates the use of traditional calibration curves and provides direct mathematical quantification of the analyte from the isotope ratios of the endogenous and spiked analyte analogues. The use of this technique can allow for more frequent patient monitoring and give physicians higher quality actionable data. In collaboration with Scott Faber MD this test may facilitate diagnosis and aide in treatment of those on the autism spectrum.
Introduction: In the biopharma QC environment, peptide mapping with LC-MS is an analytical tool utilized in lot release of Biotherapeutics. Increased throughput of this approach is required by the pharmaceutical industry. Multi-pump UHPLC systems can be configured to enable tandem analysis with two columns in parallel and thereby increase throughput. The actual run time is shortened by removing the wash and re-equilibration steps from total run time, which are carried out offline when the second column is running the separation.

Methods: A UHPLC multi-pump setup, coupled to a high resolution accurate mass (HRAM) mass spectrometer was used for the tandem LC approach. A commercially available monoclonal antibody sample was digested using an immobilized trypsin kit and separated with columns in tandem operation optimized for peptide separations. The data were acquired and analyzed with a single chromatography data system.

Preliminary data: The UHPLC systems can be easily configured for tandem LC-MS operation for peptide mapping experiments by adding one additional pump module. The developed tandem LC-MS setup enables up to 60% higher throughput without changing the actual gradient of the existing method. The retention time RSD values are below 0.11% for the tandem and single column operation. In this study, peptide mapping methods were used to demonstrate the capabilities of a tandem LC setup, but it can be applied to other methods and samples as well. The LC-MS system with a single point software control fulfills GMP/GLP requirements and is a turn-key solution for fully integrated and high throughput sample handling.

Novel aspect: Advanced system configuration, GxP complying, to boost throughput and productivity in batch release of protein therapeutics by LC-MS.

Keywords: Liquid Chromatography, Mass Spectrometry, Pharmaceutical, Protein

Application Code: Bioanalytical
Methodology Code: Liquid Chromatography/Mass Spectrometry
Amino acids are essential building blocks in all living organisms, and the identification and quantitation of amino acids from various samples are important. Recently, the liquid chromatography with tandem mass spectrometry is used as a prevalent method for amino acids analysis. Amino acids are usually derivatized to improve the detection by increasing the amino acid mass to a higher range. However, some of these LC-MS methods are based on the detection of fragment from the derivatization reagent rather than the targeted analyte, which lose specificity of the detection. A novel method based on the paired ion electrospray ionization (PIESI) mass spectrometry has been developed for the sensitive and selective detection of N-derivatized amino acids with specific fragments from derivatized molecule. Various N-derivatization methods such as 9-fluorenylmethyl chloroformate (Fmoc), 7-fluoro-4-nitrobenzoxadiazole (NBD-F) and 5-dimethylamino-1-naphthalenesulphonyl-chloride (DANSYL) were evaluated with the developed method. Fmoc derivatization showed great improvement with 1 to 3 order of magnitude more sensitive for amino acids detection when applied with PIESI in positive mode.
Application of Liquid Chromatography/Mass Spectrometry

**Abstract Title**

**LC/MS Analysis of Oligonucleotides Using New Polymer-Based HILIC Column Having Diol Group**

**Primary Author**

Leah Block
Showa Denko America, Inc.

**Abstract Text**

Highly sensitive and highly selective LC/MS analysis is required for the development or quality control of oligonucleotide drugs. Conventionally ion-pair reverse-phase chromatography have been used, unfortunately ion-pair reagents tend to remain in the LC/MS instrument. In order to solve this issue, a new HILIC column with polyvinyl alcohol base packing material modified with diol group, was used for the LC. Aqueous solutions including ammonium formate and acetonitrile were used as the eluent, coupled with ESI-MS were used for detection. As a result, good separation of 2-20mer DNA was obtained allowing the calibration curve of 20mer to show high linearity. This method enables the analysis of oligonucleotides without ion-pair reagent. This HILIC column is useful for the LC/MS analysis of oligonucleotide medicines using more simple conditions than previous methods.

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

**Application Code:** Pharmaceutical

**Methodology Code:** Liquid Chromatography/Mass Spectrometry
Peptides and other biological molecules such as proteins and monoclonal antibodies are becoming increasingly popular in many therapeutic areas such as drug discovery, medical diagnostics, and precision medicine. Because the initial stages of drug discovery often require only small amounts of target compound, fast and efficient product isolation is a key element to meeting aggressive development timelines. While traditional peptide isolation is generally performed using UV detection, mass-directed isolation makes the purification process easier with better clarity between target peptide and the contaminants formed during synthesis and cleavage. In addition, developing a separation that utilizes both mass and UV detection ensures a more complete chromatographic sample profile. Compounds that do not ionize, or ionize poorly, will often be detected with low wavelength UV. Conversely, peptides with very low UV extinction will usually be detected with mass analysis. The fluidically-optimized flow path of the UPLC combined with a specially-designed low dispersion fraction collector enable the mass-directed isolation of sharp, narrow product peaks. In this study, we illustrate the utility of the ACQUITY UPLC H-Class and Waters Fraction Manager-Analytical (WFM-A) Systems for the analysis and isolation of a synthetic peptide at the small scale. Fast, targeted isolation increases purification efficiency by reducing unnecessary sample handling while generating just enough product for future experiments.

Keywords: Biopharmaceutical, Mass Spectrometry, Peptides, Prep Chromatography
Application Code: Pharmaceutical
Methodology Code: Liquid Chromatography/Mass Spectrometry
Chiral separations have traditionally been accomplished using cellulose/amylose based stationary phases and normal-phase chromatography. Although possible with some LC-MS sources, normal-phase solvents are often detrimental to ionization in those tandem systems, which limits enantiomeric separations in areas, such as bioanalysis, where LC-MS analysis is necessary. Besides the normal-phase used with cellulose and amylose polysaccharide chiral stationary phases is not compatible with biological samples. Macrocyclic glycopeptide chiral stationary phases are alternatives, which use typical reversed phase mobile phases or polar organic solvents, such as methanol, making them highly amenable for LC-MS phases using LC-MS method. In this study, we investigated the retention and selectivity on teicoplanin and vancomycin bonded silica phases using LC-MS method. We screened a set of basic probes differing in pKa values, hydrophobicity and molecular weight to probe the impact of buffer (salt) type, buffer concentration and acid/base ratio on retention, and selectivity. We then specifically investigated analysis of several beta-blocker drugs from rat plasma using MS-compatible mobile phases. It is demonstrated the methodology can be used in bioanalysis of clinical samples. The separation conditions and results will be discussed.

Keywords: Chiral Separations, HPLC Columns, Liquid Chromatography/Mass Spectroscopy, Pharmaceutical

Application Code: Pharmaceutical

Methodology Code: Liquid Chromatography/Mass Spectrometry
Here we present a highly robust novel capillary-flow LC-MS platform that combines capillary-flow UltiMate 3000 RSLCnano system (capLC), the new 150 µm ID capillary-flow EASY-Spray column and the new Q Exactive HF-X mass spectrometer. We used typical shotgun and targeted proteomics experiments (Full-scan MS and Data-Dependent Acquisition (DDA)) to verify the performance and robustness of the novel capLC-MS platform using complex human samples: (i) HeLa cell lysate protein digest and (ii) crude plasma protein digest. We found that capillary column with 150 µm ID can be operated at a wide flow range from 1 to 3 µL/min, corresponding to linear velocities from 2 to 4 mm/s. The results with capLC-MS are 40 times more sensitive than analytical flow LC-MS at 450 µL/min and only 2 to 4 times less sensitive than nanoLC-MS at 300 nL/min (Figure A). New 150 µm ID EASY-Spray column provides excellent chromatographic performance with peak width at half max (PWHM) as low as 3 s at 3 µL/min flow rate.

Capillary-flow LC system delivers high gradient reproducibility and retention time stability with relative standard deviations (RSDs) less than 1 % for long term (7 days) operation. The coupling of capLC with Q Exactive HF-X that has brighter ion source and up to 40 Hz acquisition speed permits the identification of more than 4000 protein groups within a 60 min capLC-MS analysis cycle (Figure B). The abundance range for proteins identified in cell lysate digest as well as in crude plasma covered 4 orders of magnitude.

The novel capillary-flow LC-MS platform combines the advantages of high-throughput analytical flow LC separations and the high sensitivity of nano-flow LC-MS analysis. The high robustness and sensitivity of capLC-MS permit routine profiling of proteomics samples and quantitative analysis in large sample cohorts.

Keywords: HPLC Columns, Liquid Chromatography/Mass Spectroscopy, Proteomics, Tandem Mass Spec
Application Code: Genomics, Proteomics and Other ‘Omics
Methodology Code: Liquid Chromatography/Mass Spectrometry
The differential diagnosis of asthma and COPD can be hampered by their overlapping clinical presentation. With the absence of routine clinical tests of adequate specificity in outpatient settings, diagnosis is usually achieved through therapy trials. Metabolomics investigates all metabolites in biological systems and aims at biomarkers identification. A recent 1H-NMR study suggested 50 polar urinary metabolites as candidate biomarkers for asthma and COPD. The metabolites were divided into 3 groups. Groups 1 and 2 contain amine and carboxylic acid metabolites, respectively. They were quantified through newly validated LC-MS/MS methods using differential isotope labeling (DIL) strategies. Group 3 contains 7 metabolites of diverse chemical properties rendering them unquantifiable through DIL approach. Accordingly, a hydrophilic interaction liquid chromatography (HILIC)-MS/MS method was developed for their quantification. Stable isotope internal standards (ISs) are used for matrix effects correction. The developed method was validated according to the FDA and EMA guidelines. However, some uncommon analytical and/or statistical approaches were adopted in order to meet the encountered challenges. For instance, while the calibration samples are constructed in neat solvent, standard addition in control pooled urine sample is used for QC samples preparation. Unusual interference was also observed, for the first time, with d2-glucose in urine at 227>181 m/z. This was corrected through the use of 227>121 m/z for the IS while quantifying glucose at 225>179 m/z. Isotopic interference from 2 analytes was also observed at their ISs MRM channel.

Asthma and COPD patients’ samples were analyzed and data was combined with those obtained from groups 1 and 2 metabolites. The preliminary statistical model constructed though PLS-DA showed adequate separation between patient groups. However, more samples need to be processed before the most significant biomarkers can be finalized.

**Keywords:** Liquid Chromatography/Mass Spectroscopy, Method Development, Validation, Metabolomics

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

**Methodology Code:** Liquid Chromatography/Mass Spectrometry
Nootropic compounds are a class of chemicals that may provide cognitive enhancement to the brain by improving memory, creativity, focus, and motivation. They are sometimes referred to as “smart drugs” and include both naturally-occurring and synthetic compounds. The use of these “smart drugs” is becoming more popular among college students and people who work in competitive industries, like the technology sector. Some nootropics such as modafinil (prescribed to treat sleep disorders) have been approved by the Food and Drug Administration (FDA). Others, such as piracetam, have been approved for use in other countries, but have not gone through the approval process in the U.S. Since many of the nootropic compounds have not gone through clinical trials, their general safety and efficacy is unknown. Several of the nootropic compounds are also stimulants, including methylphenidate and caffeine, among others. Nootropic and stimulant compounds have been identified in dietary supplements marketed towards cognitive enhancement, as well as in pre-workout supplements. Over the past several years our laboratory has seen a steady increase in the number of samples that fall into these dietary supplement categories. In response to this increase, we have developed a rapid method to screen dietary supplements for over 50 different nootropic and stimulant compounds using ultra-high pressure liquid chromatography with mass spectral detection (UHPLC-MS). The method employs a gradient using 0.1% acetic acid in water and 0.1% acetic acid in methanol with a C8 column to separate the compounds in 10 minutes, with 3 additional minutes for equilibration. An ion trap mass spectrometer equipped with an electrospray ionization source is used for detection.
Application of Liquid Chromatography/Mass Spectrometry

Simultaneous Determination of Bisphenol A Derivatives in Water by LC-MS/MS

Introduction:
Bisphenol A (BPA) derivatives including bisphenol A diglycidyl ether (BADGE) and bisphenol F diglycidyl ether (BFDGE) are used in inner layer of coated cans and various kind of food packages and containers. Nowadays it is well known that both compounds (as well as their aqueous and hydrochloric derivatives) can produce a genotoxic effect in the consumers. Due to their toxicity, the European Union has established legislation concerning the specific migration limits (SML) for BADGE and related compounds in foods (Commission Directive 16/EC, 2002; Commission Regulation EC 1895, 2005). Regulation specifies maximum SML of 9 mg kg⁻¹ for the sum of BADGE and their hydrolysed derivatives (BADGE·H₂O, BADGE·₂H₂O and BADGE·HCl·H₂O) as well as 1 mg kg⁻¹ for the sum of BADGE and their hydrochloric derivatives (BADGE·HCl, BADGE·₂HCl and BADGE·HCl·H₂O). The presence of BFDGE has been prohibited since 2005.

Method:
Water samples was centrifuged and supernatant was subject to analysis. BPA and its derivatives separated by Kinetex C18 (2.1 mm I.D. x 50 mm L, 2.6 µm) by gradient elution with a flow rate at 0.3 mL at 40°C. The analysis was detected with LCMS-8050 under positive and negative electrospray ionization modes at same time and quantitation was performed using multiple reaction monitoring.

Results & conclusions:
In the present study, a rapid, sensitive and specific method was developed to simultaneous determination of BPA, BADGE·H₂O, BADGE·₂H₂O, BADGE·HCl·H₂O, BADGE·HCl, BADGE·₂HCl and BFDGE in water by ultra-performance liquid chromatograph-tandem mass spectrometry (LC-MS/MS). The results showed that the linear correlation coefficient of 8 BPA and its derivatives were bigger than 0.996 from 0.2 ng/mL to 100 ng/mL with the repeatability of 2.92%~10.19% (0.2 ng/mL , n=6).

Keywords: Beverage, Contamination, Liquid Chromatography/Mass Spectroscopy, Water

Application Code: Food Safety

Methodology Code: Liquid Chromatography/Mass Spectrometry
Urinary diamines are potential biomarkers of environmental exposure to their corresponding diisocyanate; the diamines of interest include 1,6-hexamethylenediamine (HDA), isophoronediamine (IPDA), p-phenylenediamine (PPDA), 2,6-toluenediamine (6TDA), 2,4-toluenediamine (4TDA), 1,5-naphthalenediamine (5NDA), and 4,4-methylenediphenyldiamine (4MDA). ß-Methylamino-L-alanine (BMAA) forms as a result of human exposure to blue-green algae contaminated food. And, trimethylamine N-oxide (TMAO) is excreted in urine due to dietary intake of carnitine and choline rich diets. These urinary biomarkers represent a class of small nitrogen-containing compounds with wide-ranging logP and pKₐ values that is difficult to separate under common chromatographic techniques, such as HILIC and reversed phase. We report on the development of an ultraperformance liquid chromatography-electrospray ionization-tandem mass spectrometry (UPLC-ESI-MS/MS) based method that affords simultaneous measurement of this challenging class of urinary biomarkers using ion-pairing reagents. Results show baseline separation of all analytes within 5 minutes with ion-pairing reagents heptafluorobutyric acid and pentafluoropropionic acid. Using this method, it is possible to quantitate the analytes to low ng/mL concentrations. Future work will provide further analytical figures of merit, including accuracy, precision, and robustness. The method will be developed for biomonitoring studies within targeted groups as well as larger population-based studies such as the US National Health and Nutrition Examination Survey (NHANES). Please note that the funding for this work is from the funding allocated to the Oak Ridge Institute for Science and Education (ORISE).
Over the years, the Forensic Chemistry Center (FCC) of the United States Food and Drug Administration (FDA) has received numerous requests to analyze syringes/needles, human tissue, and unknown liquids for the presence of polydimethylsiloxane (PDMS) that was injected into patients for cosmetic enhancement by unlicensed individuals. While infrared spectroscopy/infrared spectroscopic imaging are the primary methods for identifying PDMS in these samples, headspace gas chromatography with mass spectrometric detection (HS/GC-MS) has been successfully utilized as a complementary/secondary method for the detection of low molecular weight volatile cyclic siloxanes (CS), which are considered impurity markers for PDMS. However, thus far, the HS/GC-MS method has only been applied to the detection of low molecular weight (LMW) CS (<500 Da) such as hexamethylcyclotrisiloxane (D3), octamethylcyclotetrasiloxane (D4), decamethylcyclopentasiloxane (D5) and dodecamethylcyclohexasiloxane (D6). Lately, efforts have been focused in the development of methods with soft ionization sources (e.g. liquid chromatography with mass spectrometry, LC-MS) to detect both LMW and high molecular weight (HMW) CS.

In this study, LC-MS with electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) sources were evaluated for monitoring LMW CS (D3-D6) and HMW CS tetradecamethyl cycloheptasiloxane (D7), hexadecamethylcyclooctasiloxane (D8) and octadecamethylcyclononasiloxane (D9). For the LC-MS experiments, an Ion Trap mass spectrometer was evaluated. Chromatographic separations were performed utilizing a reverse phase column. Depending on the conditions used, the generated precursor ions were protonated or ammoniated. LC-APCI-MS was found to be the preferred method for the detection of CS, where it provides a sensitive technique capable of detecting LMW (D3-D6) as well as HMW (D7-D9) CS.

**Keywords:** Consumer Products, Forensics, Liquid Chromatography/Mass Spectroscopy

**Application Code:** Consumer Products

**Methodology Code:** Liquid Chromatography/Mass Spectrometry
As a complex solid sample matrix, hair requires pretreatment measures, including washing, homogenization, and isolation of drug from hair matrix components, to allow for analysis. While numerous methods exist for forensic hair analysis, they have not been systematically compared with regard to performance, thus providing the objective for this work. Drug (amphetamine) was incorporated into purchased human head hair for use as reference material. Design Expert (StatEase) was used to establish the definitive screening design used for the systematic comparison of pretreatment parameters. Each run varied the values for numeric and categorical pretreatment parameters systematically to ultimately determine the optimal conditions for maximizing recovery of drug. Quantitative analysis of each sample was conducted using an Agilent 1290 Infinity HPLC System coupled to an Agilent 6460 triple quadrupole mass spectrometer. The recoveries from each run were compared and optimized with response surface modeling. Base degradation of the hair matrix resulted in the highest recovery of incorporated amphetamine from hair (60%). The DoE studies for this extraction indicated that optimized recovery may be achieved using a hair sample of 35.8 mg homogenized into a powder and washed with 1% sodium dodecyl sulfate and methanol. For optimized recovery, the ratio of extraction solvent to hair sample should be 12.6, and the incubation should occur for 16.4 h at 48°C. When expanded to include additional drugs, it is expected that this research will contribute to determining the most effective method(s) for isolating drugs of abuse from hair samples for routine analysis in forensic laboratories.

**Keywords:** Forensic Chemistry, Liquid Chromatography/Mass Spectroscopy, Toxicology

**Application Code:** Other

**Methodology Code:** Liquid Chromatography/Mass Spectrometry
**Abstract Text**

Various chiral amino alcohols, e.g. (R)-phenylglycinol, (S)-leucinol, (S)-alaninol, N-3,5-dinitrobenzoyl amide derived chiral stationary phases (CSPs) were synthesized and used for the resolution of various chiral analytes. As a result, (R)-phenylglycinol derived CSP (CSP 1) performed better than others. The (R)-Phenylglycinol was also used as a starting material of new C3 symmetric (R)-phenylglycinol N-1,3,5-benzenetricarboxylic acid derived chiral stationary phase (CSP 2) and new C2 symmetric CSPs (CSP 3 ~ CSP 5) published on Chirality1. A new C3 symmetric (R)-phenylglycinol N-1,3,5-benzenetricarboxylic acid derived chiral HPLC stationary phase (CSP 2) and the CSP 1 showed good results in comparing with previously reported N-3,5-dintrobenzoyl(DNB) (R)-phenylglycinol derived CSP.

In this study, new C3 symmetric CSPs(CSP 6 ~ CSP 8) were newly prepared by the ideas and results of the three mentioned references2,3. The synthetic procedures and applications of the new C3 symmetric CSPs (CSP 6 ~ CSP 8) will be shown.

**Keywords:** Chiral Separations, Chromatography, HPLC  
**Application Code:** Other  
**Methodology Code:** Liquid Chromatography/Mass Spectrometry
**Introduction**

Proteinogenic amino acids (except for glycine) have a chiral center at alpha carbon atom adjacent to the carboxyl group and form two enantiomers. L-Amino acids are present in the body as a component of proteins and nutrients in large quantities. On the other hand, D-Amino acids are extremely low, but functions such as central nervous system and hormone secretion control are being elucidated \[\text{i}n \text{vivo}\]. Also, they are drawing attention as a functional ingredient in foods.

Here we developed the method that be possible to analyze chiral amino acids in just 10 minutes using a chiral column without derivatization and confirmed ratio of D/L amino acids in fermented foods. And we also developed the column switching system to achieve high-throughput comprehensive analysis of D/L amino acids.

**Methods**

Separation was achieved using CROWNPAK CR-I(+) / CR-I(-) (DAICEL Corporation) on a HPLC system (Prominence, Nexera X2, Shimadzu Corporation). Data acquisition was performed on triple quadrupole mass spectrometer LCMS-8050 / 8060 (Shimadzu Corporation). The mobile phase consisted of a mixture of acetonitrile, ethanol, water and TFA (80/15/5/0.5) and the flow rate was set to 0.6 mL/min.

We investigated automatically analysis system using valve switching units for continuously analyzing CROWNPAK CR-I(+) and CR-I(-). A mixture of 22 amino acids and the fermented foods are used as samples.

**Results**

A mixture of 22 amino acids were detected in 10 minutes by using CR-I (+)/CR-I (-). Then we confirmed ratio of D/L amino acids in black vinegars and yogurts. A relatively large amount of D-Ala, D-Arg, D-Asn, D-Glu, D-Lys and D-Ser were included.

**Conclusions**

We achieved high-throughput comprehensive LC-MS/MS analysis of D/L amino acids with chiral column and column-switching system. The system is effective for analysis of chiral amino acids in food sample.

**Reference**


**Keywords:** Amino Acids, Chiral Separations, High Throughput Chemical Analysis, Liquid Chromatography/Mass S

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

**Methodology Code:** Liquid Chromatography/Mass Spectrometry
Application of Liquid Chromatography/Mass Spectrometry

Qualitative and Quantitative Analysis of Contaminants of Emerging Concern in Biosolids Using UHPLC-High Resolution Orbitrap Mass Spectrometry

Contaminants of Emerging Concern (CECs) are generally described as contaminants that were unknown, unrecognized (not detectable) or unregulated (not routinely monitored). They represent a diverse group of chemicals such as pharmaceuticals, personal care products, endocrine disrupting agents, and biological metabolites. Many of these compounds are likely present in wastewater treatment plants effluents because wastewater treatments were not specifically designed to remove them. Thus, a portion of CECs could be present either in a dissolved form in water or adsorbed onto sewage sludge. The treated sewage sludge (biosolids) are frequently used as an inexpensive agricultural fertilizers. Land-applied biosolids can introduce CECs into soil and water and this may pose potential threats to human health and the environment.

This work presents a sensitive, selective and reliable ultra-high pressure liquid chromatography-high resolution mass spectrometry (UHPLC-HRMS) method for the analysis of CECs in biosolids. Samples were extracted using ultrasonic solvent extraction with no sample clean-up. This method has been optimized for the determination of 52 CECs present in biosolids and terrestrial biomes exposed to biosolids amended soils. Knowing the concentrations of CECs in biosolids and biological tissues would allow for the assessment, where appropriate, of the uptake and bioaccumulation. In addition, full scan HRMS data provides information on the possible environmental transformation by-products for possible environmental accumulation and ecological effects that would not be available with other available technology.

Keywords: Contamination, Environmental Analysis, Liquid Chromatography/Mass Spectroscopy
Application Code: Environmental
Methodology Code: Liquid Chromatography/Mass Spectrometry
Since the inception of electrospray ionization (ESI), method development and optimization has been an ongoing and challenging task. It can be difficult even for experts in the field, as well as for novices and newcomers to LC-MS and IC-MS techniques. Although the body of knowledge on both the analytes and the solvents/additives/matrices has grown, generating a new “from scratch” analytical ESI method remains largely a trial-and-error process for the novice. Creating and optimizing an ESI method is further exacerbated by the interdependencies of the method parameters and intrinsic variability between ion sources (both from different instrument models, and even more so between different manufacturers). Heated electrospray (HESI) method optimization cannot be reduced to partial derivatives in the method parametric space and remains a tedious task even for the experts in the field, despite the fact that such optimization is routinely and frequently required in the lab.

We will present our solutions for simplifying the method optimization process by (a) eliminating the source position variability and complexity, (b) decreasing dependence upon tuning ion optics, (c) introducing algorithms that interlink the method parameters with each other based upon the desirable analytical figures of merit (d) compartmentalizing tuning variables into categories based upon physico-chemical characteristics of the analytes and the mobile phase, that are intuitive to the user, and (e) wrapping those algorithms into an easy-to-adopt and easy-to-use graphical user interface of the ISQ EC instrument method editor that allows novices not only to create a working HESI method from scratch but also educates them on the individual method parameters values and brings to the next level of expertise in IC-MS and LC-MS.

Keywords: Instrumentation, Ion Chromatography, Liquid Chromatography/Mass Spectroscopy, Method Development

Application Code: Consumer Products

Methodology Code: Liquid Chromatography/Mass Spectrometry
The impurity profile understanding of active pharmaceutical ingredients (API) and excipients plays an important role in drug development, limit content of related impurities as well as in quality control. Mass spectrometry independent data analysis experiments (MS IDA) offer significant advantages for the characterization of unknown impurities specially in a complex matrix such as lipids extracts that combined with the understanding of the nature/origin and possible mechanism of impurities formation become a powerful tool for impurities identification. Myristic acid was tested using Rapid Resolution Liquid Chromatography (210 nm, UV detector) coupled to quadrupole time-of-flight mass spectrometry (QTOF MS). Untargeted chromatographic profile showed several unsaturated and hydroxylated fatty acids (FA) that were not detected using a GC-FID-MS method. MS IDA experiments in -ESI and +ESI mode provided further structural evidence of the additional impurities observed by UHPLC-UV and results will be shown. The relation of double bond location and conformation to the affinity to a UHPLC C18 column, MS/MS fragmentation profile and understanding of naturally occurring FA shortened the list of potential detected impurities. The most intense impurities detected at 210 nm were purchased and used to assign a relative response factor that provided a simple quantitative method for organic impurities profile by LC-UV in myristic acid. The FA 14:1 cis-[delta]9 and cis-[delta]5 (Nu-chek/TRC) were co-injected in order to confirm impurities identification. Purchased 3-hydroxy myristic acid (Sigma-Aldrich) was used as reagent to synthesize FA 14:1 trans-[delta]2 and trans-[delta]3 through water elimination reaction using base which were used to further confirm the identity of two additional impurities. The FA 14:1 trans-[delta]2 and trans-[delta]3 were proposed to be potential by-product formed during the myristic acid extraction/concentration steps.
We present the use of underwater laser induced breakdown spectroscopy (LIBS) to detect two rare earth elements; Eu and Yb dissolved in bulk aqueous solutions at ambient and high-pressure conditions. Strong emission lines of Eu and Yb were identified in the underwater LIBS spectra. Temporal evolution of plasma, the effect of laser pulse energy, and the effect of pressure on the spectral emission were studied. Calibration curves were developed to demonstrate the possibility of using LIBS technique for their quantification and limits of detection were estimated.
Spectral line assignment from databases in optical emission spectroscopy requires a high level of confidence for quantitative analysis. Line broadening and instrumentation effects degrade the resolution of spectra resulting in spectral interferences that occur for the majority of the lines. Spectral interferences create uncertainty in the elemental profile.

This presentation will cover the application of a developed factor that quantifies the level of confidence to line assignment in LIBS. This factor couples the physical understanding of plasma emission with statistical analysis of the spectrum.

Applications for pure samples as well as NIST glass standards will be shown and discussed.

The work presented is funded by the US National Institute of Justice (2012-DN-BX-K027: “Level of Confidence in Elemental Analysis by LIBS”) and the State of Florida.
In glow discharge spectrometry, one could examine Werner Grimm’s 1967 lamp geometry and modern glow discharge instruments and observe that much has stayed constant with the source, which remains the standard for glow discharge analysis across manufacturers. Despite centuries of experimentation with gas discharges, sampling solid materials for spectrochemical analysis with a glow discharge remained a challenge until Grimm’s invention of a new lamp configuration. Grimm designed a lamp that allowed observation of the discharge on-axis with a lamp that sampled in a remarkably reproducible way with even sputtering across the surface. Samples could be quickly loaded and unloaded by altering the vacuum conditions and the lamp window was kept clean over time. These advantages persist in modern instrumentation.

Many lamp features were optimized and changes enabled new types of analyses. Grimm’s six-millimeter discharge area was shrunk to four to obtain more even electric fields and reduce power requirements without sacrificing sensitivity. A reamer-facer assembly was developed to clean the lamp between samples and hold the sample in place during analysis. The lamp was modified for analysis of non-conductive materials with radio-frequency power. Today, a light flow of argon near the lamp orifice inhibits ambient gas from entering the cell, keeping the argon atmosphere more pure. Compositional depth profiling brought about the need for fine control and monitoring of voltage, current, and pressure for accurate calculations of sputtering rate and optimal crater shapes. Depth profiling of nanometer-scale features at the near-surface of modern materials enables rapid thin-layer analysis. With emerging applications and capabilities, the future of glow discharge spectroscopy is anything but grim.

Keywords: Atomic Emission Spectroscopy, Elemental Analysis, History of Chemistry, Instrumentation
Application Code: Material Science
Methodology Code: Atomic Spectroscopy/Elemental Analysis
Analysis of Used Engine Oils Using Laser-Induced Breakdown Spectroscopy (LIBS)

Engine oil analyses can help maintain engine health and prevent engine failure before it occurs. Laser-induced breakdown spectroscopy (LIBS) can be used for engine oil analyses by determining the metal concentrations in used engine oil samples. In this work, the atomic emission lines of Al, Cr, Fe, Mg, and Mn were used to build the Partial least squares regression (PLS-R) calibration models for the determination of the Al, Cr, Fe, Mg, and Mn concentrations in used engine oils. We also used the PLS-R calibration model to determine the limits of detection (LODs) for the Al, Cr, Fe, Mg, and Mn lines. The LODs for the Al(I) 394.4 nm, Cr(II) 427.48 nm, Fe(I) 438.35 nm, Mg(I) 285.21 nm, and Mn(II) 259.37 nm lines were 0.75, 1, 0.8, 0.43, and 0.74 ppm, respectively. By analyzing the used engine oil, we can predict engine part problems with high concentrations of a specific metal found in the used engine oil. C[2]/nm content in engine oil could be an indicator for the engine oil degradation. In this work, we had used the C[2]/nm molecular band emissions to determine the degree of engine oil degradation. A decrease in the peak intensity of the molecular carbon band, the C[2]/nm (0,0) band at 516.5 nm, indicates increased metal concentrations and engine oil degradation.

Keywords: Calibration, Plasma Emission (ICP/MIP/DCP/etc.), Spectroscopy
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Atomic Spectroscopy/Elemental Analysis
The requirement for Sulfur determination at very low levels (few ppm) is getting more and more important, for both quality control and basic research purposes in different applications fields such as petrochemistry, environmental, agronomy, marine science, food and animal feed, and material characterization. Chemical composition of geological samples are tightly connected with the origin of earth matter and often serve as indicators of geological processes while for materials are tightly connected with the specific characteristic and their properties. Specifications and legislative limitations on hazardous and toxic pollutants from vehicle exhaust emissions have been introduced due to increased atmospheric pollution. Developing countries are working towards reducing the sulfur content in gasoline and diesel. Monitoring key elements at lower concentration is routinely applied to exploration programs, research and environmental projects and is strictly controlled to meet national and international regulations to avoid potential danger due to hazardous and toxic substances. Many of the classical methods for sulfur determination are no longer suitable for routine analysis. Analytical instruments based on the combustion of samples improve the reliability of the data available to industry, without the use of hazardous chemicals. Total Sulfur contents can be accurately determined by the FlashSmart Elemental Analyzer coupled with a Flame Photometric Detector (FPD). The method combines the OEA advantages with sensitivity, selectivity and robustness of FPD. This paper presents Sulfur data of different solid and liquid matrices in trace concentrations analyzed several times to show the reproducibility obtained with the system.
Elemental analyzer with a thermal conductivity detector for Nitrogen and Carbon determination uses as typical Helium carrier gas due 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 FlashSmart Elemental 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 EagerSmart dedicated Data Handling Software for the quantification of the Nitrogen and Carbon content (NC) in simultaneous or as only Nitrogen analysis. This paper presents data on NC and N determination of several matrices in a large range of concentration in order to demonstrate the performance of the instrument using Argon gas in terms of accuracy and repeatability.

Keywords: Elemental Analysis, Optimization, Quantitative, Soil
Application Code: General Interest
Methodology Code: Atomic Spectroscopy/Elemental Analysis
Carbon, Nitrogen, Hydrogen, Sulfur by combustion analysis is very commonly used for the characterization of raw and final products in all application fields for quality control and R&D purposes. The use of an accurate and automatic analytical technique which allows the fast analysis with an excellent reproducibility in all range of concentration is required. The FlashSmart™ Elemental Analyzer operating with the dynamic flash combustion of the sample (modified Dumas method) with the Thermal Conductivity Detector (TCD), meets laboratory requirements, such as accuracy, reproducibility, day to day reproducibility and high sample throughput. The Analyzer is equipped with two totally independent furnaces allowing the installation of two analytical circuits which are used alternatively and completely automatic through the MultiValve Control™ (MVC) Module. Each analytical circuit can receive its own autosampler. Through its flexibility, the analyzer allows also trace Sulfur determination, when the Analyzer is coupled with the Flame Photometric Detector (FPD). Accordingly to these features, the Analyzer can be configured for CHNS determination on the left furnace with TCD detector and for trace sulfur analysis on the right furnace with the FPD detector. This paper presents CHNS and trace sulfur data of pure organic standards, reference materials and several matrices to evaluate the accuracy and precision of the data using different configurations and detectors.

Keywords: Automation, Elemental Analysis, Quantitative, Sulfur

Application Code: Material Science

Methodology Code: Atomic Spectroscopy/Elemental Analysis
In recent years, the use of laser-induced breakdown spectroscopy (LIBS) has increased markedly, although the low sensitivity of some LIBS instruments has limited somehow its application. To overcome this problem a simple and low-cost strategy, i.e. laser-induced electrical discharge (LIED) has been introduced for reheating the plasma and so increasing the LIBS signal intensity. The aim of this work was to develop a LIED-LIBS system for P determination in inorganic fertilizers. Powdered and dried samples (masses around 0.3 g) were compacted in a hydraulic press. The homemade assembled LIBS system consisting of a Q-switched Nd:YAG laser operating at 1064 nm and emitting pulses at 50 mJ with a fluence of 70 J cm\(^2\) at the focal point, a sample chamber, optical fibers and a spectrometer with optical resolution of 0.1 nm (full width at half maximum). The instrumental parameters selected to monitor P (I) at 214.9 nm were 1-ms integration time and 1-µs Q-Switch delay. Two cylindrical W electrodes (100 x 2.6 mm) were arranged with tips at a distance of 4 mm and 2 mm above the sample surface. The homemade high-voltage electronic circuit provided an output voltage in the 0 – 4.5 kV range. Standards (4.8, 11.9, 22.9, 27.8 and 33.34% P\(_2\)O\(_5\)) were prepared by mixing a SRM-120c phosphate rock reference material with CaCO\(_3\), CaSO\(_4\), (NH\(_2\))\(_2\)CO and KCl high-purity salts at a 1:1:1:1 mass ratio. Although no emission at 214.9 nm was observed using classical LIBS, the use of the LIED-LIBS at 4.5 kV output allowed obtaining calibration curves with correlation coefficients \(0.993\), RSD \(8\%\) and a LOQ of 5.3% (m/m) P\(_2\)O\(_5\). Contents of P in six samples determined by the LIED-LIBS system were in good agreement with those obtained by HR-CS FAAS. Besides increasing the sensitivity for P, the LIED option is simple, of low-cost acquisition and maintenance, of easy operation and adjustable to any LIBS system, thus it appears very promising for application to other analytes and matrices.
An automatic flow-through dynamic system with high temporal resolution and elucidation of the bioaccessible elemental fraction of food commodities in the gastrointestinal tract is herein proposed for the first time for exploring the transit of chyme from the gastric to the duodenal compartments based on the Oomen’s fed-state physiologically relevant extraction method. The flow manifold was coupled on-line to inductively coupled plasma optical emission spectrometry (ICP OES) for real-time multielemental analysis of bioaccessible fractions of micronutrients (Cu, Fe and Mn) in well-defined volumes of extracts (300 [micro]L) of commercially available golden and brown linseed. The simulated intestinal and bile biofluid (added to the gastric phase) was successively pumped at 1.0 mL min\(^{-1}\) across a large-bore column (maintained at 37.0 ± 2.0 [degree]C ) initially loaded with a given amount of weighed linseed sample (250 mg) onto a PVDF filter membrane (5.0 [micro]m pore size). Due to the lack of consensus in the literature regarding the agitation method in oral bioaccessibility testing, several batch agitation approaches (viz., magnetic stirring, end-over-end rotation and orbital shaking) were evaluated. Magnetic stirring was chosen because improved extractability was observed and the experimental results were comparable to the dynamic counterpart based on the continuous displacement of the extraction equilibrium. Using this agitation method, the volume of saliva + gastric phase was optimized so as to equate batch with dynamic extraction results. The trueness of the on-line gastrointestinal extraction method as a front end to ICP OES was confirmed using mass balance validation following microwave assisted digestion of the residual (non-bioaccessible) elemental fraction and the original sample. Mass balance validation yielded absolute recoveries ranging from 77 to 116% regardless of the target element and sample.
Itata Valley, located in the South Valley, comprises an area bordered by the Andes and a lower coastal mountain, and it is one of the most important winemaking areas in Chile. Current trends regarding winery practices imply the production of ‘natural wines’, wines made without chemical and minimum technological intervention in growing grapes, and also in winery practices. Chemical characterization of these wines is scarce, and data regarding bioavailability of essential, and also toxic compounds, are not available.

The aim of the current work has been the assessment of the bioavailable fraction of trace and ultratrace metals in Chilean ‘natural wines’. An in vitro bioavailability approach based dialyzability has been applied. The in vitro digestion procedure consisted of two sequential stages, which simulate gastric and intestinal digestion. The first step requires 0.15 g of a freshly prepared gastric solution [6.0 %(m/v) pepsin in 6.0 M hydrochloric acid], and incubation at 37 ºC with an orbital – horizontal shaking at 150 rpm for 120 min. The second step uses 5.0 mL of intestinal solution (4.0 %(m/v) pancreatine and 2.5 %(m/v) bile salts dissolved in 0.1 M sodium hydrogen carbonate), and also incubation at 37 ºC with an orbital – horizontal shaking at 150 rpm for 120 min]. During this second stage, dialysis membranes (10 kDa cut-off) filled with 20 mL of 0.15 N PIPES (pH 7.5) were used for simulating bio-absorption. Total concentrations of trace and ultratrace metals in wines were directly assessed by inductively coupled plasma – mass spectrometry (ICP-MS) after 1:10 dilution with 1.0 % (v/v) nitric acid. Similarly, dialysates analysis has also been performed by ICP-MS after 1:5 dilution in ultrapure water. Bio-availability ratios (dialyzability) were finally obtained by calculating the percentages of total metals concentrations in the PIPES solution inside the dialysis membranes (bio-available fraction), and those found in wines.

Keywords: Beverages, Food Science, ICP-MS
Application Code: Food Science
Methodology Code: Atomic Spectroscopy/Elemental Analysis
Multivitamin (MV) supplements are very complex samples containing heavy metal and radionuclides impurities originating from the materials used in manufacturing. Major elemental components of MV supplements include boron, calcium (Ca), chloride (Cl), magnesium (Mg), phosphorus (P), potassium (K), iron (Fe), manganese (Mn), copper (Cu), and zinc (Zn) and various proteins. The concentrations of heavy metals, such as arsenic (As), cadmium (Cd), lead (Pb) and uranium (U) are usually in sub-µg/g. Accurate quality control at such levels by ICP-MS is a challenging task due to the complexity of MV supplements. Certain elements added as nutrients induce spectral and chemical interferences. Chloride causes ArCl interference on As. Tin (Sn) isotopes has direct overlap on Cd isotopes, while various molybdenum oxides (Mo) exhibit isobaric overlaps on Cd isotopes. Removal of MV matrix components is important to eliminate interfering components and alleviating the adverse effects of MV matrix on ICP-MS analysis. In this study, we developed a co-precipitation approach for selectively separating As, Cd, Pb and U from MV matrix. Sequential co-precipitations using trimethylamine (TEA) and hydrofluoric acid (HF) were performed. Proteinaceous and inorganic matrices (B, Ca, Mg, Cl, Fe, K and K) were successfully removed. Sn matrix eliminated to extent. Mo levels reduced from 400 ppm to 0.02 ppm in analysis solutions alleviating the spectral interferences on Cd. The method was applied to the determination of As, Cd, Pb and U in multivitamin certified reference material (SRM 3280) then applied to the analysis of various commercially available MV tablets.

Keywords: Elemental Mass Spec, Method Development, Separation Sciences, Ultratrace Analysis
Application Code: Food Safety
Methodology Code: Atomic Spectroscopy/Elemental Analysis
The results of a survey of selected elements (Fe, Cu, Zn, Ca, Cr, Pb, Al, Cd, Hg, As, Na, K) in tap water from selected buildings on the campus of the University of Pittsburgh – Greensburg will be presented and discussed in this paper. This study was inspired by the recent incidences of lead contamination in the municipal water supplies of Flint, Michigan and Pittsburgh, Pennsylvania. In fact, many municipalities across the United States have had problems with contamination of water used for human consumption by toxic metals or metals that, while non-toxic, can still render water supplies unusable. Not only are municipal water supplies susceptible by toxic metals contamination, well water from springs on private lands can be potentially contaminated by such threats as runoff from abandoned coal mines. The EPA as well as state and local regulatory agencies have set allowable limits on the concentrations of various toxic and some non-toxic metals in water supplies designated for human consumption. Thus, the focus of this study is to determine if there are levels of toxic and other relevant metals in the tap water of the Greensburg campus that are cause for concern, as per the allowable limits for each metal in drinking water set by federal, state and local regulatory agencies. The hypothesis is that Pitt-Greensburg tap water will contain mostly Na, K, Ca, and Mg ions, with lower concentrations of Fe and Zn, while the remaining metal ions to be determined will have concentrations below their detection limits.

Sample collection and preparation procedures will be presented and discussed, as will the determination of each element selected by flame atomic absorption spectrometry (FAAS). The results obtained, and their significance, as well as future directions for this study, will be presented and discussed.

Keywords: Atomic Absorption, Detection, Metals, Water
Application Code: Environmental
Methodology Code: Atomic Spectroscopy/Elemental Analysis
Rapid Determination of Trace Elements in Serum by ICPMS-2030 with Collision Cell

Reference pre-processing method in the standard method of WS-T-93-1996 “Determination of copper in Serum by Atomic Absorption Spectrometry”, serum samples directly diluted with dilute acid, a method to determine concentrations of Ca, Cu, Fe, Mg and Zn elements in serum was studied by Inductively Coupled Plasma-Mass Spectrometry (ICPMS-2030 of Shimadzu) with collision cell. The method detection limits were 17~300 µg/L. The proposed method was validated by the serum reference materials Seronorm™ Trace Elements. The results were consistent with certified values. The determination precision (RSD, n=6) of the serum samples were less than 4%, and the relative standard deviation of parallel sample was less than 1.4%. This method has a low detection limit with high precision and accuracy, all of which meet the requirements to analyze serum samples.

Keywords: Biological Samples, Elemental Analysis, ICP-MS
Application Code: Bioanalytical
Methodology Code: Atomic Spectroscopy/Elemental Analysis
Atomic Spectroscopy/Elemental Analysis

Determination of Inorganic Arsenic in Melarsomine Dihydrochloride Active Pharmaceutical Ingredient

Melarsomine dihydrochloride is an organic arsenical chemotherapeutic agent used as an API for the treatment of canine heartworm caused by Dirofilaria immitis. As part of a FDA requirement the API has to contain less than 250ppm of inorganic Arsenic based on the summation of Arsenate and Arsenite. The test is challenging as the API contains 14% by mass of Arsenic which can affect the measurement technique by causing contamination and carryover between samples. Chromatographic separations were developed to not only separate Arsenate and Arsenite but also to flush the API agent from the column. Method Development and validation was conducted using Ion chromatography with Hydride Generation – Atomic Fluorescence Spectrometry. A summary of the validation data according to VICH GL2 guidelines will be presented. Validation of the method included a detailed study of system suitability, specificity, linearity, accuracy, precision, robustness, limit of detection, limit of quantification and ruggedness.

Keywords: Atomic Spectroscopy, Method Development, Pharmaceutical, Speciation

Application Code: Pharmaceutical

Methodology Code: Atomic Spectroscopy/Elemental Analysis
Atomic Spectroscopy/Elemental Analysis

Simultaneous Determination of 23 Metal Elements in Hair By ICP - MS

Introduction
The human body contains a variety of essential, non-essential and harmful trace metal elements. Accurate detection of these trace elements is conducive to guide people's dietary structure, control the ion balance of body fluid, and protect the health of the body. Biological materials that can be used for detection include saliva, body fluids, urine, blood, hair and tissue. Hair trace element test results have many difficult control factors, but because it can reflect a certain time period of changes, and has the advantages, including easy to collection and transport, suitable for any age of the crowd and so on. Hair has been applied in a variety of analysis and research. Inductively coupled plasma mass spectrometry (ICP-MS) can simultaneously determine the content of various metal elements, with high sensitivity and low detection limit.

Methods
In this paper, the method of the content of 23 metal elements in hair samples was determined by inductively coupled plasma mass spectrometry (ICP-MS-2030) combined with microwave digestion. ICP-MS is performed with external calibration covering the elements of interest in concentrations comparable to the samples. For quality control of the sample preparation procedure, internal standards such as Germanium (Ge), Scandium (Sc), Indium (In) and Bismuth (Bi) can be included in each sample digestion, spiked to the acid used.

Results & Discussion
The method is applied to the analysis of metal content in the standard material. The linear correlation coefficient is good, r > 0.9998. The experimental results are in good agreement with the standard values, and the method is accurate and reliable. This method has the characteristics of high sensitivity, low detection limit, easy to operate, and provides a useful reference for the determination of metal elements in human hair samples.

Keywords: Bioanalytical, ICP-MS
Application Code: Bioanalytical
Methodology Code: Atomic Spectroscopy/Elemental Analysis
Portable Energy Dispersive X-ray Fluorescence (pXRF) spectrometers have been commercially available field tools for effective elemental screening and analysis of materials since the late 1970s. Portable handheld XRF analyzers utilizing tube-based sources, SiPIN and SDD detectors introduced in the early 2000’s dramatically expanded their applicability to well beyond the measurement of heavy metals in alloys and soil or Pb in paint. pXRF is now a staple analytical tool for positive material identification in manufacturing and scrap recovery, natural resource exploration, environmental & soil science, consumer safety, art conservation, archaeometry, precious metals, forensics, education & research, advanced materials and, more recently, in food safety and agriculture.

Although handheld EDXRF is highly applicable, there are many situations where portable, open beam x-radiation is not wanted or even permitted. Many of the transportable benchtop EDXRF systems available are simply too bulky and/or cannot be battery powered to be considered truly field portable. This presentation will demonstrate the utility of a new, light-weight, compact and self-contained closed-beam pXRF that is truly field-portable and applicable to all handheld XRF analyses other than in-situ measurements.

A full description of this new product development will be provided, including its x-radiation source, detector, optical geometry, speed, sensitivity and elemental range. The user interface, including operation, measurement parameters, interactive communication and data analysis software will also be described.

Data presented will include measurements for natural resource exploration, MARPOL sulfur in fuel regulations and agricultural analysis. Best practices in methods and sample presentation will be illustrated.

Keywords: Elemental Analysis, Environmental Analysis, Portable Instruments, X-ray Fluorescence
Application Code: Other
Methodology Code: X-ray Techniques
Total carbon and sulfur determination in refractory materials is a common analysis that has been historically performed with resistance or induction furnace instruments. Total carbon and sulfur combustion instruments use a high temperature furnace to achieve complete decomposition of the sample material. The carbon and sulfur present in the refractory materials are liberated as CO2 and SO2, respectively. The combustion gases are swept through the instrument’s reagents and carried to infrared detectors, where the infrared absorbance of CO2 and SO2 are measured and converted to a quantifiable concentration based on initial sample mass.

Many factors influence which furnace style is optimal, such as water content (free and crystalline), the presence of organic binders, and decomposition temperature. Instruments with resistance furnaces are better suited for the analysis of materials with elevated moisture or organic content, but require the use of promoters to achieve full sulfur recovery. Induction furnace instruments are better suited for the analysis of materials with high decomposition temperatures, and also require the use of combustion promoters. New resistance furnace technology with elevated analysis temperature capabilities, up to 1550°C, allow for new methods in the analysis of refractory materials without the use of combustion promoters.

This poster presentation will compare the results of total carbon and sulfur determination by various elemental analysis techniques featuring both resistance and induction furnace instruments, with and without the use of combustion promoters. Data will be examined that includes refractory materials such as lime, limestone, glass, cement, and other industrial raw materials.

Keywords: Analysis, Elemental Analysis, Method Development, Thermal Analysis
Application Code: Other
Methodology Code: Thermal Analysis
The development of SI traceable measurements for the monitoring and control of mercury in gas emission sources and in the atmosphere is of prime importance for various groups, including mercury researchers, plant operators, regulators and also for the implementation of the Minamata Convention. Currently traceable calibration systems only exist for elemental Hg however such requirements are also required for oxidized Hg species namely (Hg²⁺). Knowledge of Hg speciation in both air and in stack emissions is critical when validating models for predicting Hg emissions, transportation, deposition and fate on a global scale. Numerous methods and instrumentation exists for gas phase Hg speciation although this is rarely calibrated for oxidized Hg and if so the metrology of the calibrator is unknown. This raises the uncertainty of the speciation measurement and also inhibits the development of new measurement protocols. The MercOx project is a European funded EMPIR Metrology project to develop traceable Hg online measurements for gas emissions and the atmosphere. In this paper we will provide an overview of our research work on the development and metrological assessment of various oxidised Hg calibration systems. Approaches include liquid evaporative HgCl₂ generators, HgCl₂ permeation devices, dilution of saturated sources of HgCl₂ at known temperature, catalytic oxidisation of elemental Hg and ultra-sonication of aqueous HgCl₂ solutions. These approaches will be coupled to various wet chemical, sorbent trap and Hg-CEM mercury speciation measurement technologies.
Due to the diversity of aerosols, there exist large variations in morphology, height to diameter ratio (aspect ratio, AR), and mechanical properties (Young’s modulus, YM) that change based on chemical composition, phase state, and relative humidity (RH). Although these parameters are important and may dictate how the aerosols can affect the climate and the atmosphere, current experimental methodologies cannot directly measure all of them at once. Herein, we demonstrate the ability to directly measure the 3D morphology and mechanical properties by using atomic force microscopy (AFM) for the atmospherically relevant sea spray aerosol (SSA) particle model systems. The mimics studied here contain both organic and inorganic systems as both homogeneous and binary mixtures. Using nanoindentation capabilities of the AFM, we also show that our methodology can categorize the systems into solid, solid-like and liquid-like phase states by analyzing the force profiles obtained during this study from low to high RH. From our measurements, most of the single component inorganic systems showed higher ARs and YMs at 20% RH compared to single component organic systems. Organic-inorganic binary chemical mixture showed different ARs and YMs which was in between their individual ARs and YMs. High ARs correlated positively with high YMs, attributed to the significant contribution of height from the core, which is often comprised of crystalline salts with high YM. Overall, this study establishes the methodology that reveals strong correlation between ARs and YMs of the SSA model systems. The correlation can be described using a polynomial function that can potentially be used to characterize nascent SSA solely by imaging to quantify the AR, and be able to predict the YM.

Keywords: Atomic Spectroscopy
Application Code: Environmental
Methodology Code: Atomic Spectroscopy/Elemental Analysis
Total joint replacement arthroplasty (TJA) has physically enabled and liberated thousands from debilitating hip and knee diseases or injuries. In the long term, TJA might be associated with adverse local and remote tissue responses, and that these effects may be mediated by the degradation of prosthetic materials. To study the systemic distribution of corrosion and wear products from TJA in the human body, the metals of TJA devices must be accurately measured in blood and tissues. NIST in collaboration with Centers for Disease Control and Prevention (CDC) is developing SRM 955D Toxic Metals and Metabolites in Frozen Human Blood. One of the intended use of the SRM is providing method validation for accuracy in monitoring of metals of corrosion from TJA. Chromium, one of the elements from metal-on-metal type TJA device, is to be certified in SRM 955D. Chromium is very difficult to measure because it appears in the blood at sub ng/mL level. Inductively coupled plasma mass spectrometry (ICP-MS) is the most sensitive technique for Cr measurement; however, the most useful Cr isotopes at masses 52 and 53 suffer isobaric interference from diatomic ions 40Ar12C+ and 40Ar13C+, respectively. In addition, the ubiquity of Cr makes the measurement susceptible to contamination. The paper describes a dilute-and-shoot approach to minimize the contamination and to circumvent the isobaric interferences by measuring Cr as an oxide for the accurate isotope dilution determination of Cr in blood using triple-quadrupole ICP-MS (ID-QQQ-ICP-MS).

**Keywords:** Clinical Chemistry, Elemental Analysis, ICP-MS, Quadrupole MS

**Application Code:** Clinical/Toxicology

**Methodology Code:** Atomic Spectroscopy/Elemental Analysis
Selenium has been recognized as an essential micronutrient for humans and its deficiency is frequently associated with different kinds of cancer including prostate cancer. It is presented in naturally occurring food mostly in the selenomethionine form, which is also considered one of the most bioavailable forms of selenium. Since selenium content in food is highly dependent on the amount of selenium presented in the soil, which can vary from country to country and even from region to region within a country, the levels of selenium available in human diet in certain geographic areas can differ significantly. As a result, the use of selenium supplementation, mainly as selenium-enriched yeast, has become very popular in the last few years. Due to this fact, methodologies for the characterization of not only the dosage of selenium present in those supplements but also the particular form are of extremely importance. Separation techniques hyphenated with ICP-MS has been widely used for speciation of metals. Chromatography is the most known form or separation technique but it can cause structural disruption and denaturation of the protein chain due the high force of interaction between the analyte and the chromatographic column. Field flow fractionation (FFF) can be used as an alternative method to chromatography since allows an interaction between the analytes and the field sufficient to separate them, but is not extensive enough to occur degradation.

FFF is suitable for separation of macromolecule, supramolecule and particulate analytes in a very broad molar mass range and holds great promise for the analysis and characterization of protein aggregates and particles, due its wide dynamic range and matrix-free separation mechanism. This works presents the applicability of FFF coupled to ICP-MS for speciation of selenomethionine in supplements. Several extraction procedures were evaluated and method validated with the analysis of a CRM for selenium enriched yeast.
Time and labor should not be wasted for manual work that can be done by automated processes. Very often manual sample preparation can be handled by automated Gel Permeation Chromatography (GPC cleanup). Further, reproducibility and quality of your sample is improved. The dedicated system processes up to 15 samples while requiring minimal bench space. The cleanup process can be performed by the AZURA GPC cleanup system which is operated by touch-optimized Mobile Control.

GPC Cleanup (Gel Permeation Chromatography) is primarily employed for performing general cleanup tasks on a wide range of sample matrices such as foodstuffs, tissues, plants and environmental samples. The separation of components takes place according to their molecular weight due to the heterogeneous pore size of the stationary phase. High-molecular substances such as lipids, proteins, natural resins, cellular components and steroids interfering with subsequent analysis e.g. of pesticides are efficiently removed.

The AZURA GPC Cleanup system is operated with Mobile Control. This clearly arranged user interface is run on a tablet directly mounted at the system. It automatically recognizes devices and facilitates system configuration. Due to block programming methods are created fast with a minimal number of clicks. Mobile Control makes sample loop loading easily manageable by synchronously switching both valves at the push of a button. The software Mobile Control provides an intuitive and cost-effective user interface for device control directly at the system.
The roasting of the coffee is an unitary operation which allows fixing and formation of composite chemical aromatic, characteristic of the processed grains and which are dependent on the same speed of the process. The are both changes in the color, density and porosity. This work has studied the evolution of parameters chromatic and physicochemical properties of beans during the roasting process. Arabica coffee (Coffea arabica L. var. Caturra), were subjected to slow roasting using toaster semi-industrial at 160 °C. Chromaticity parameters were determined at later stage to the process, in samples collected at intervals of 1 min. for luminance (L), red/green (a*), yellow/blue (b*), chromaticity, chroma (C), hue (h) and color difference (ΔE) with respect to the fresh coffee changes. The standard illuminant (D65) reference, the area of measurement of 0.95 cm². Additionally was determined (ρ) porosity, real and aparent density (ρ), (\(\rho\)). Wide and long (l cm) beads. During roasting the grains present morphological and structural variations in size and shades of color (CIELab), (\(\rho\)), (\(\rho\)) and (\(\rho\)). The final phase, is exothermic and proceeds after the breakdown of the grain. The observed changes in L drop from 40.8 ± 0.42 to 31.2 ± 0.78 a* (0.75 ± 0.21 at 6.65 ± 0.21) and b (10.2 ± 0.02 at 8.60 ± 0.04) (yellow-green, to Brown). The angle of pitch (h) silent 85.8 ± 0.84 to 52.3 ± 0.63. Respect (\(\rho\)), the parameter changes of 658 to 386 kg m\(^{-3}\)), (\(\rho\)) is reduced by 24% (672-508 kg m\(^{-3}\)). The greatest effect of roasting, generates an increase in volume of grain, with weight loss, reduction of moisture and density. It has shown the temperature and time of exert a significant effect on quality and physicochemical properties of the grains.

Keywords: Food Science, Identification, Process Monitoring
Application Code: Food Science
Methodology Code: Physical Measurements
One of the most important nutrient in animal nutrition is protein. Protein intake provides the building blocks needed by the animal to produce its own body proteins in order to grow. The protein needs are to be very specie/phase targeted to reduce excess and avoid deficient amino acids as well as reducing the amount used for energy when not necessary. The precise and accurate determination of its amount, through the determination of Nitrogen, is fundamental to achieve the nutritional quality of animal finished products. For these reasons, the capabilities of the Dumas method (combustion method) for the determination of Nitrogen have been greatly improved to make faster, safer and more reliable than the traditional Kjeldahl method. Combustion Dumas method has been approved and adopted by the Association of Official Analytical Chemists (AOAC Official Method 990.03 Protein crude in Animal Feed 4.2.08). In this light, the FlashSmart Elemental Analyzer, based on the dynamic flash combustion of the sample, cope with a wide array of important requirements of laboratories such as accuracy, day by day reproducibility and high sample throughput. However as in the last years there was large cost increasing for Helium, it is necessary to test as alternative gas, Argon which is readily available. This paper presents data on Nitrogen/Protein determination of raw and final animal products with different Nitrogen concentration to show the performance of the system using Argon gas and the reproducibility of the results obtained in comparison with the values obtained using helium as carrier gas.
The purpose of this experiment is to quantitatively determine the content of Raffinose Family Oligosaccharides (RFOs) (stachyose, verbascose and raffinose) in variety types of pulses. This oligosaccharides family is important in promoting gut health, since the bacteria in large intestine is able to produce a necessary enzyme, β-galactosidase to breakdown their complex structures and use the product for fermentation, in which increases the growth of colonies. RFOs content in pulses were determined using the Megazyme Raffinose/ D-galactose assay.

The assay has three main enzymes (β-galactosidase, galactose mutarotase and β-galactose dehydrogenase) to convert RFOs to NADH. The absorbance of the product was measured using UV spectrophotometer at 340nm. In this reaction, the amount of NADH produced was stoichiometrically comparable with the amount of RFOs converted. The enzymes efficiency was 91.3±0.2% when measured with the raffinose control. This method was used to determine RFOs content in soybeans, lentils, chickpeas and adzuki beans. Soybeans had the highest RFOs content 0.594±0.01 g/L among the selected pulses. Different solvents such as pure water, 90% methanol, 95% ethanol, 80% ethanol containing 1M HCl, and 58% acetonitrile were used to obtain the optimum solvent conditions for the RFO extraction. The water extraction gave the highest RFO content and water was selected as the best extraction solvent. Finally, RFOs prebiotic powder was added with increments of 2%, up to 10% w/v into a yogurt starter culture, and stored at 4°C. Physicochemical characteristics, such as pH, titratable acidity and syneresis were monitored over a duration of 21 days, and then compared with inulin prebiotic added yogurt and the control sample.
Food Science

Modernization of the USP Dietary Supplement Monograph for Boswellia Serrata with Advancements in HPLC/UHPLC Column Chemistry

Frankincense is gaining interest as a dietary supplement, and Boswellia serrata is one of the main botanical species that contribute to its efficacy which can be adulterated or mislabeled with other species. The current USP monograph effectively quantifies 4 of the 6 characteristic Boswellic acids with a traditional, fully-porous, 5 um C18 HPLC column in a 45 minute run-time.

A collaborative work with the USP, Alkemist Laboratories and Extrasynthese was done to optimize a core-shell technology HPLC/ UHPLC column and a range of column phase chemistries were utilized for optimizing a method that produces run-times in less than 10 minutes and is flexible across HPLC and UHPLC platforms. The method includes the two additional alpha boswellic acids to effectively quantify all 6 characteristic boswellic acids. These improvements in chromatographic technologies are readily accessible and parallel the needs of the Food Safety Modernization Act (FSMA), that is aimed at a scientific approach to meet safety and quality requirements for the global food supply.

Keywords: HPLC, HPLC Columns
Application Code: Food Science
Methodology Code: Liquid Chromatography
Increased resolution and efficiency are every analytical chemist’s desire. Though a 60 minute analytical run has traditionally been common for analysis of fatty acid methyl esters (FAMEs) due to their unique structures, analysts in production environments often mention the need for faster run times to improve productivity. Using several key GC column parameters including selectivity and dimensions, run time for a typical 37 component FAMEs sample can be reduced to as short as 12 minutes. Using a high-cyano Zebron™ ZB-FAME GC column, demonstration of successful tactics for achieving short, successful FAMEs testing methods for real samples is explored. Multiple matrices including food and fuel were explored. For much complex matrix, a 2D GC approach was also considered with two complimentary selectivity GC stationary phase ZB-FAME and ZB-5MS+.
Vitamin Ds are essential human nutrients responsible for increasing intestinal absorption of calcium, iron, magnesium, phosphate, and zinc. There is a concern that the intake of vitamin D among some population groups is inadequate. Recently, the FDA required that vitamin D content must be declared on all food labels(1). The analysis of vitamin D in food often involves saponification, extraction, clean-up, and HPLC. One of the challenges in vitamin D analysis is the interference from the sample matrix. Recent developments in vitamin D analysis include the use of a derivatization agent, 4-phenyl-1,2,4-triazole-3,5-dione, or PTAD, to simplify the sample prep, to reduce the interference, and to increase sensitivity for vitamin D analysis. However, the existing vitamin D analysis methods that using PTAD derivatization do not measure the previtamin Ds. Vitamin Ds can reversibly isomerize to previtamin Ds. The previtamin Ds and vitamin Ds are both biologically active. The relative content of the previtamin Ds can be significant. It is necessary to measure the previtamin Ds in addition to the vitamin Ds in foods to get an accurate vitamin D result. In this study, the use of PTAD for the vitamin D and the previtamin D analysis by UPLC-MS is investigated.
Acid Beverage Floc (ABF) is a visual defect that can occur in sugar sweetened acidified beverages, typically carbonated soft drinks. This defect manifests itself as a white wispy precipitate similar in appearance to a cotton ball and arises from impurities present in the sugar ingredient. If a sugar is to cause ABF, it can take up to 10 days to form, often after the beverage has been shipped to retailers for sale. Whilst ABF is harmless and tasteless to the consumer, it results in the rejection of the product by the customer purely for aesthetic reasons.

With the commercial value in managing ABF for their products, beverage companies often require sugar refiners to provide no-floc guarantees for their product. This high purity sugar, termed “bottlers grade”, requires additional testing for the sugars ABF propensity. The standard method for ABF analysis is specified by the International Commission of Uniform Sugar Analysis (ICUMSA Method GS 2/3-40) and simply involves the preparation of a simulated beverage using the sample sugar to observe if ABF forms. The standard has two major drawbacks; an excessive analysis time of 10 days and providing only a qualitative measurement. The lack of a rapid and reliable method for testing ABF in cane sugar is due to the impurity responsible for its formation being unknown.

In this study, a galactoglucomannan (GGM) has been isolated from both authentic ABF and cane sugars from Australia. This polysaccharide has been identified as the impurity responsible for the initiation of ABF in soft drinks that use Australian cane sugar. The GGM is sparingly soluble in sugar solutions leading to the formation of micro flocs in the beverage. Other sugar impurities such as starch, protein, silica and waxes then adsorb onto these micro flocs to produce the observed ABF. A structure for the GGM has been proposed using a combination of GC-FID, GC-MS and NMR techniques.

This research was supported by an Australian Postgraduate Award scholarship.

**Keywords:** Beverage, Carbohydrates, Characterization, GC-MS

**Application Code:** Food Science

**Methodology Code:** Gas Chromatography/Mass Spectrometry
Many of the flavors and fragrances which make up a wine's profile consist of volatile organic compounds (VOCs). These chemicals, even at small concentrations can affect the flavor and aroma of a wine. Many subjective descriptors are used such as buttery, a hint of oak, peppery, vanilla, and so on. Wine flavors and aromas can be organized into three primary groups: fruit, floral; and herbal, spice, and earth. VOCs are produced at different times in the winemaking process. For instance, VOCs accumulate in the grape as the grape seed matures and as the fruit ripens by binding to other molecules such as sugars and amino acids. Winemakers release these compounds by breaking the bonds: both physically by crushing the grapes and chemically during fermentation with grape and yeast enzymes. Traditionally the detection of VOCs has been accomplished by the oenologist through taste and smell. There is growing interest in using chemical analysis to identify and quantitate the VOCs in the various stages of the winemaking process. For example, esters can add fruit and flower notes; terpenes can add piney, rose, and lavender notes; and organic acids can add sour, vinegary notes. The use of GC/MS may lead to identification of previously unknown VOCs in wine as well as help winemakers make adjustments to the growing of wine grapes and winemaking techniques based upon scientific data.

Many VOCs can be analyzed by purge and trap concentration and detection by GC/MS. A variety of wines will be analyzed by this technique. A calibration will be run using commercially available compounds and a library search will be performed on peaks not identified by the calibration method, i.e., tentatively identified compounds (TICs).

**Keywords:** Beverage, Gas Chromatography/Mass Spectrometry, Purge and Trap, Volatile Organic Compounds

**Application Code:** Food Science

**Methodology Code:** Gas Chromatography/Mass Spectrometry
Flavour Analysis of Milk and Dairy Products Using a Novel Sorptive Extraction Technique with Thermal Desorption Pre-Concentration and GCMS Analysis

Milk, as a liquid product produced on a vast scale, is highly susceptible to contamination – from chemicals used in agriculture, from animal feed, or from the transport, processing and packaging processes. The flavor composition of milk and dairy products is complex and based on a broad range of both VOC and SVOC compounds. Reliable analysis of the volatile components of milk is therefore valuable to ensure high quality. Traditionally, solid phase micro-extraction (SPME) has been used for flavour profiling. It is a fast and simple technique, but can be limited in terms of sample capacity, reproducibility and sensitivity. Here we utilise high-capacity sorptive extraction for immersive sampling of milk using a novel design of sampling probe. The robust, high-capacity PDMS phase results in higher sample loadings than SPME methods, and combined with pre-concentration by thermal desorption (TD) offers greater sensitivity across a wider analyte range with no requirement for solvent extraction or other sample preparation. The new design of sorptive extraction probe provides a simple to use but effective technique for the analysis of a range of milk and aqueous based milk products. Increased sorptive stationary phase loading and immersed extraction enables a greater level and range of compounds to be identified from each sample. Combining this extraction technique with Thermal Desorption preconcentration optimizes the sample preparation process for maximum sensitivity.

Keywords: Extraction, Food Safety, GC-MS, Thermal Desorption

Application Code: Food Science

Methodology Code: Gas Chromatography/Mass Spectrometry
Malted barley is one of the main ingredients used in brewing beer. It provides complex carbohydrates and sugars that are crucial for fermentation and it also imparts flavor, body, and color to the final product. The flavor contribution depends largely on the style of malt used. Base, caramel, and roasted malts are all common and each lends unique flavor notes and characteristics. A chew test of whole kernels has traditionally been used as a check for flavor and freshness. While this test provides insight and has the benefit of being very fast, it does not fully reflect the flavors that are extracted during the brewing process that would be anticipated in the final product. Recently, a Hot Steep Method has been developed to prepare a malt extract for sensory analysis that is more representative of the malt in the beer. In this work, we aim to further investigate these malt extracts with a non-targeted chemical analysis. A variety of malts were prepared according to the hot steep method. The extracts were sampled with head space solid phase micro-extraction (HS-SPME) to collect the volatile and semi-volatile flavor analytes and then subsequently analyzed with gas chromatography coupled to mass spectrometry (GC-MS). This data provided information on individual analytes present in the samples. Many of the observed analytes have known flavor and odor properties which can be connected with the sensory attributes of the malts.

Keywords: Beverage, GC, GC-MS, Time of Flight MS
Application Code: Food Science
Methodology Code: Gas Chromatography/Mass Spectrometry
The pervasion of our everyday life by smart mobile devices has led to unprecedented possibilities in terms of communicating, socializing and information gathering. Due to their mostly open programming interfaces, their high processing power as well as their inbuilt connectivity features and sensors, mobile smart devices offer the potential for the development of diverse applications not only for the private, but also for the professional sector. Wearables like smartglasses provide new opportunities in areas with special requirements, like laboratories, and supply essential advantages over handheld smart devices. Experiment and device parameters can be displayed directly in the experimenter’s field of view and integrated voice recognition capabilities ensure a control in accordance with hygiene and safety regulations.

The developed application shows the use of smartglasses within the food laboratory. Different standard operating procedures (SOPs) for the spectrophotometrical analysis of beer, in accordance to the regulations of the Central European Commission for Brewing Analysis, were structured into successive handgrabs and visualized. In the following these working steps were implemented into a voice-triggered Android SOP application which is capable to establish a bidirectional communication with a photometer that is involved in the analytical processes. All arising parameters, working steps and results are documented in real-time and saved inside a protocol document on a server within the laboratory network, which can be accessed later on. The established digital laboratory infrastructure ensures a flexible addition of new standard operating procedures and the integration of additional laboratory devices to the existing setup. The developed solution is not only limited to analytical applications in food laboratories and might be of special interest for education and training purposes and for the use in laboratories with recurring tasks.
The production of fragranced consumer products is subject to a number of areas where it is important to understand the impact of VOCs and SVOCs contained within a product. From a product stewardship perspective, there is increasing focus on emissions of volatiles under normal use conditions, for example during the burning of a fragranced candle, with ongoing new developments in regulations for many products used in indoor environments. Equally, from a marketing perspective, consumer product manufacturers are continually working on new formulations which present a pleasant, long-lasting aroma which will be attractive to consumers. Aroma profiling in this industry is not limited to new product development, but also in quality control to investigate any reports of poor product quality or malodours. Traditional methods for aroma & fragrance profiling include static / dynamic headspace and solid-phase microextraction, for studying both raw materials and final products. In this study, dynamic headspace sampling and high-capacity sorptive extraction are employed to a range of different fragranced household products. These high-capacity, versatile sampling techniques are combined with TD, the inherent pre-concentration capability of which aids detection of low concentration, aroma-active compounds whilst also having the capability to deal with higher concentration analytes. Using multiple sampling techniques also results in more extensive sample characterisation than would be achieved with a single sampling approach.

Keywords: Extraction, Food Identification, GC-MS, Quality Control
Application Code: Food Identification
Methodology Code: Gas Chromatography
The determination of cis/trans fatty acid methyl esters (FAMEs) is a nice solution for some label claim and grading requirements in Olive Oil. As its popularity continues to increase, due to reported health effects, and culinary uses, further profiling is necessary for a quantitative authenticity test, especially with different organoleptic regional notes. Profiling sterols by GC can help quantify these otherwise subjective, but important consumer properties, while assisting from regional adulteration/label claim fraud.

Several official methods for detecting adulterants and determining olive oil quality have been established in order to guarantee authenticity and safety. One such method is the determination of sterol concentrations in olive oil. A sterol profile provides an important criterion for distinguishing virgin olive oil from refined oil as well as a fingerprint for the detection of several seed oil adulterants and even geographical origin. Solid Supported-liquid extraction (SLE) using a Strata DE (diatomaceous earth) column followed by solid-phase extraction (SPE) cleanup with an activated Strata Si-1 cartridge was used to determine the concentration sterols, erythrodiol and uvaol in olive oil. Sterol profiles obtained with this method confirmed the classification of adulterated and certified extra virgin olive oils according to International Olive Oil Council (IOC) criteria. Method accuracy was assessed from the absolute recoveries of brassicasterol (82%), campesterol (75%), stigmasterol (77%), -sitosterol (100%), erythrodiol (134%) and uvaol (118%). Compared to the official IOC procedure, which uses liquid-liquid extraction and thin-layer chromatography, the SLE-SPE modification is less time-consuming and labor-intensive, avoids the use cumbersome glassware and large amounts of solvent, and allowed up to 16 samples to be extracted in parallel.
Pollen grains are microscopic structures found in the anthers of stamens in angiosperms. According to the regulations of honey and other bee products. This natural product that worker bees collected in nature adding to its own specific matter which forms the pellets, and place them in the honeycomb cells. Special group of worker bees that collect pollen is called corbicular bee Pollen. Flower pollens' composition can vary due to their botanical and geographic origin. Some studies show us that the polyphenol composition of pollen, can be a factor in its determination. Chromatic properties (CIELab) and profiles of HPLC-DAD of alcoholic extracts were probe for flavonoids and phenolic compounds. 30 samples of bee pollen from Boyacá, Colombia were analyzes. A C18 column (4.6 x 250 mm, 5 p.m.) was used in the separation process. Acidified water (pH 2.5/orthophosphoric acid) (A) and acetonitrile (B) was uses as elution mixture following linear gradient: starting with A 100%, decreasing to 91% at 12 min, 87% 20, 67% 32 and 57% at 42 min, and maintaining that level up to 60 min of analysis. The flow rate was 0.8 mL/min at room temperature. All chromatograms were recorded at 260 and 340 nm. Spectral data for all peaks were recorded in the range from 220 to 400 nm, using a diode array detector. The structural information was obtained by direct comparison of retention times and Spectra UV, each compound resolved in chromatograms with the corresponding to the reference compounds. A total of 23 phenolic acids, and 16 flavonols, and three dihydroflavonoids were found. Quercetin glycosides were the predominant compounds in the Colombian bee pollen from highland Andean zones of Boyacá.

**Keywords:** Agricultural, Analysis, Array Detectors, Bioanalytical  
**Application Code:** Food Identification  
**Methodology Code:** Liquid Chromatography
Major isoflavones that are primarily found in soy (Glycine max), red clover (Trifolium pretense), and Kudzu (Pueraria lobata) are daidzein, glycitein, genistein and their respective glucoside derivatives. Methods for the determination of isoflavones in dietary supplements have been established (1, 2). These standard methods are based on reversed-phase high performance liquid chromatography (RP-HPLC) and ultraviolet and visible light (UV-Vis) spectroscopy. Because of the structural similarity and the large number of isoflavones, the method chromatographic run times are too long (more than 70 minutes). In this study, we shortened the analysis time using an ACQUITY H-Class UPLC system. A single quadrupole mass spectrometer, Waters QCAUITY Mass Detector, was used to assist the method transfer and quantification. The benefits of the MS and the method performance characteristics will be presented.

Keywords: HPLC, Liquid Chromatography, Natural Products, UV-VIS Absorbance/Luminescence
Application Code: Food Identification
Methodology Code: Liquid Chromatography/Mass Spectrometry
Selenium-Enriched Pink Oyster Mushrooms ([i]Pleurotus Djamor[/i]): Evaluation of the Bioaccumulation and Influence on Proteins and Se-Proteins Distribution

The consumption of pink oyster mushrooms ([i]Pleurotus djamor[/i]), should be highlighted in human nutrition, due to high nutritional value and ability to accumulate essential elements, such as selenium (Se). Therefore, in this work was evaluated: (1) Se-enriched mushrooms production; (2) effects of the enrichment on the proteins and Se-proteins distribution. Mushroom cultivation was carried out in glass containers containing 20 g of substrate (sugarcane bagasse, rice bran, wheat bran, and CaO) inoculated. Fungi were placed at 18°C and 80% air humidity under the prevailing dark conditions for 12 days. Cultivation of mushrooms was accomplished with deionized water (control group) or solutions of Na[sub]2[/sub]SeO[sub]3[/sub]. For enrichment, a volume of 5 mL of Se(IV) solutions at different Se concentrations, in function of the substrate mass, (6.4; 12.8; 25.4 mg kg[sup]-1[/sup]) was added to the inoculated substrate. The quantification of proteins (albumins, globulins, prolamins and glutelins) and Se (total and Se-proteins) was done by Bradford’s method and graphite furnace atomic absorption spectrometry (GF AAS), respectively. The enrichment with different Se(IV) concentrations did not alter the morphological characteristics and biological efficiency (2.7 to 3.0%). Comparing the control and enriched groups, it was verified that occurred a decrease and an increase of globulins (82%) and glutelins (10%) concentrations, respectively. It was observed an increase in the total Se concentration, ranging from 27±1 µg g[sup]-1[/sup] (medium with 6.4 mg kg[sup]-1[/sup] of Se) to 77±5 µg g[sup]-1[/sup] (medium with 25.4 mg kg[sup]-1[/sup] of Se). Lastly, Se is mainly associated to albumins (9.5±0.7 µg g[sup]-1[/sup]) and glutelins (11.1±0.4 µg g[sup]-1[/sup]). These results demonstrate the ability of these fungi to absorb and bioaccumulate Se, favoring the formation of Se-proteins that are more bioaccessible species. ACKNOWLEDGMENT: FAPESP(2017/05009-7 and 2015/15515-2)

Keywords:  
Elemental Analysis, Food Science, Spectrophotometry

Application Code:  
Food Science

Methodology Code:  
Atomic Spectroscopy/Elemental Analysis
An accurate determination of moisture content in food and feed products provides important information related to food quality and safety (texture, taste, microbial stability), and is a key variable used to calculate a product’s purity, yield, and/or resulting constituent analysis on a dry basis.

One of the most common direct moisture determination methods is loss on drying using an air oven where the sample is weighed before and after exposure to an elevated temperature within an oven with air ventilation. The resulting sample mass loss is calculated as moisture.

AOAC direct moisture determination methods utilizing the air oven loss on drying technique are widely used in the food (AOAC; method 925.10) and feed (AOAC; method 930.15) industry. A new automated thermogravimetric moisture instrument (TGM800) has the ability to meet the AOAC method sample mass, oven temperature, and air flow requirements while providing additional benefits such as increased work flow efficiency, minimal operator time, and sample batch throughput of up to 16 samples with sequential sample mass measurements occurring throughout the analysis process ensuring weight constancy at the end of analysis.

This poster presentation will compare the determination of direct moisture loss on drying between an automated thermogravimetric moisture instrument (TGM800) and an air oven. The comparison will include food matrix sample data measured with the TGM800 and air oven techniques following the AOAC method 925.10 conditions and feed matrix sample data following the AOAC method 930.15.

Keywords: Agricultural, Food Safety, Food Science, Thermal Analysis
Application Code: Food Science
Methodology Code: Thermal Analysis
Eragrostis teff is an Ethiopian native grain with high nutritional value. In Brazil, this grain is grown in the Central West region and its importance in agribusiness has been increasing in recent years. However, there are few studies about its nutritional composition and biological effects. The aim of this study is to analyze the fatty acid profile of E. teff and to check its antimutagenic potential. A sensitive, simple, and reliable method of analysis with derivatization technique using fluorescent tag MPAC-Br (3-[4-(Bromomethyl)phenyl]-7-(diethylamino)coumarin) was developed with the ability to detect fatty acid profiles in teff seeds. The separation and quantification of MPAC-Br derivatized long chain fatty acids was carried out using HPLC with gradient elution and detection with laser-induced fluorescence (LIF) at 405 nm. Oil was extracted using hexane at 60°C in a Soxhlet modified system. Enzymatic hydrolysis with Lipozyme TL (Thermomyces lanuginosus) was performed to obtain free fatty acids from teff seeds. Antimutagenic activity was performed using Salmonella/microsome assay in co- and pre-treatment procedures performed using doxorubicin (DOX), 4-nitroquinolin N-oxide (4-NQO), methylmethanosulphonate (MMS) without S9 mix and aflatoxin B-1 with S9 mix. The fatty acid profile was as follows: palmitic acid (C16:0), 15.69%; stearic acid (C18:0), 6.55%; oleic acid (C18:1), 28.78%; linoleic acid (C18:2), 42.54% and linolenic acid (C18:3), 6.44. The seed flour hydroalcoholic extract showed antimutagenic activity against 4-NQO in the co-treatment in TA98 and against DOX and MMS in the pre-treatment in TA100. In the presence of S9 mix, the antimutagenic activity was evidenced in both strains in the pre-treatment procedures. In conclusion, teff seeds showed protective effects against frameshift and base pair substitution mutations which are correlated with teff's chemical composition and the profile of fatty acids indicated high percentage of polyunsaturated acids.
Production of diesel fuels has very strict guidelines, including fuel transportation from the production plant to the terminals. The guidelines guarantee that the products from the same source conform to the set standards. Continued monitoring of the quality of fuels is significant for motor engine efficiency, for environmental concerns in the case of spills, and for public security in the case of those who use the fuels to make homemade explosives. However, various companies have ownership of diesel fuels, once they leave the production site, as exemplified by BP, Shell, Chevron, Exxon, Quick Trip diesel fuels, and others. The question is, are these diesel fuels different? Can they be class identified? Quick, nondestructive and cost-effective analytical methods can be of significant service to help class identification of fuels. Diesel fuel brands have further been complicated in their chemical composition by blends involving biodiesel and ethanol mixtures. Instead of using the standard, elaborate gas chromatography-mass spectrometry technique and other involved methods of measuring physico-chemical parameters, we have attempted to class identify brands of diesel fuel using nondestructive mid-infrared spectroscopy coupled with Chemometrics. Our preliminary results show that while the diesel fuels show identical spectra and high correlation coefficients (>0.97), principal component analysis and partial least squares discriminant analysis are able to class identify the brands. New data, to be discussed in this poster, will also show that blends of diesel, biodiesel, and ethanol can be identified and analyzed by these spectroscopy-based techniques coupled with chemometrics.
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**Corrosion continues to be of great importance to the petroleum refining industry due to its impact on profitability and safety. Organic halides can contribute significantly to corrosion of pipelines and machinery, as they tend to form hydrochloric and hydrofluoric acids in refinery processes. While organic chlorides do not normally occur naturally in crude oil, they may be introduced from chlorinated solvents used for dewaxing pipelines or other equipment operations. A collaborative project with industry partners in ASTM Committee D02 on Petroleum Product, Liquid Fuels, and Lubricants to develop a new test method for the determination of organic chloride content in crude oil has recently been completed featuring combustion ion chromatography (CIC) detection. This work demonstrates the utility of this method and contains exemplary data collected from an inter-laboratory study associated with its development. Results obtained from a variety of crude oil specimens obtained from various drilling locations and spiked with multiple sources of organic chloride at low part-per-million concentrations will be presented.**

| Keywords: | Ion Chromatography, Petroleum |
| Application Code: | Fuels, Energy and Petrochemical |
| Methodology Code: | Liquid Chromatography |
Acid Number of Crude Oils and Petroleum Products by Catalytic Thermometric Titration using ASTM D8045

ASTM Standard D8045 helps refineries and labs analyzing acidity in difficult crude oils and petroleum products by using a thermometric technique that gives improved precision, faster analysis times and reduced solvent requirements. The development of this new standard solves solubility and precision challenges encountered with traditional potentiometric techniques. With over 8 years of development work in ASTM and thousands of sample data points, this new standard shows very close data comparisons between methods and encourages the use of proven innovation for safer and more precise acidity analysis.

Keywords: Petroleum, Titration
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Thermal Analysis
Electrocatalytic Oxygen Evolution Reactions on Pt Sphere and Ir-modified Pt Sphere Electrodes

Abstract Text
Oxygen evolution reactions (OER) have received recent attention because of its importance in electrochemical water splitting for sustainable power sources. Although there have been intensive investigations on the compositional optimization of metal based OER electrocatalysts, less attention has been paid for the effect of electrode structures on the OER activities. In this presentation, we investigated the electrochemical OER activity at porous Pt sphere electrodes prepared by electrodeposition. The dependence of OER activity on the porosity of the Pt sphere electrodes was systemically examined by changing the charges during the electrodeposition of Pt spheres. We also investigate the effect of nanoscale surface features on the OER activity, wherein rough Pt spheres exhibited enhanced OER activities compared to the smooth ones. The surfaces of the Pt sphere electrodes were modified with Ir by spontaneous replacement reactions, which resulted in an OER activity enhancement relative to the Pt sphere electrodes. The structure-dependent OER activity demonstrated here gives insight into the fabrication of efficient OER electrocatalysts.

Keywords: Electrochemistry, Electrode Surfaces, Electrodes, Fuels\Energy\Petrochemical
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Electrochemistry
The sluggish oxygen evolution reaction (OER) is an important half-reaction of the electrolytic water splitting reaction. Thus, efficient and economic OER electrocatalysts are of interest to overcome the kinetic barriers for the OER at the anode. In general, metal oxides (such as RuO$_2$ and IrO$_2$) have been utilized as the catalysts for OER. However, these metal oxide often suffer from disadvantages, such as scarcity, high cost and poor stability. Among many cheap and abundant transition metals, nonprecious and cheap cobalt (Co) has been reported to have a relatively good OER activity. Nanomaterials of bi-metal and/or metal oxides have attracted attention due to their new physical and chemical properties derived from synergistic effects.

In this presentation, we demonstrate the synthesis of various mixed oxide nanocomposites consisted of both Ir and Co (Ir$_x$Co$_{1-x}$O$_y$, 0 < x < 1) via subsequent electrospinning and calcination. Electrospinning is a simple and versatile technique to fabricate diverse compositions of one-dimensional nanomaterials. The physical properties of the prepared Ir$_x$Co$_{1-x}$O$_y$ nanotubes are characterized by field-emission scanning electron microscopy, transmission electron microscopy and X-ray photoelectron spectroscopy. The change of structure occurred with blending of two elements can be interpreted through X-ray diffraction and Raman spectroscopy. Electroactivity of Ir$_x$Co$_{1-x}$O$_y$ nanotubes for OER is characterized with rotating disk electrode voltammetry in 1 M NaOH aqueous solution. The OER activity of Ir$_x$Co$_{1-x}$O$_y$ nanotubes is systematically studied and interpreted as a function of the relative ratio of Ir to Co.

This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT& Future Planning (NRF-2017R1A2A2A14001137).

**Keywords:** Electrochemistry, Fuels\Energy\Petrochemical, Materials Characterization, Material Science

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

**Methodology Code:** Electrochemistry
In this work, we utilize a bipolar electrode (BPE) array to obtain a visual voltammogram in a microfluidic device. The microfluidic device is made up of two parallel microfluidic channels interconnected by a linear array of gold BPEs with chromium tips, in an arrangement resembling a ladder – the two ends of each BPE contacting separate channels. While BPEs have been employed previously for voltammetry, the arrangement reported here allows for a discreet voltage drop across each BPE. This feature is significant because it allows both thermodynamic and kinetic information to be assessed. The authors would like to extend our gratitude to Iowa State University for start up funds supporting this work.
Methane number (MN) is a measure of the quality and knock resistance of natural gas. This value is comparable to octane number used for gasoline powered vehicles. For most internal combustion engines, a methane number above 80 should be maintained to resist engine knocking and ensure full engine output. However, lower MN can be tolerated while maintaining full engine output, especially if real time engine fuel analysis is performed. Over the years there have been many ways to calculate methane number as each IC engine manufacturer developed a method that worked for their specific engines. At their core, they rely on accurate component concentrations to calculate the methane number of the natural gas. Historically, technologies for measuring natural gas composition have been based upon gas chromatography. While gas chromatography can give accurate component concentrations, it suffers from slow response time. With natural gas being transferred around the world as LNG and injected into local distribution pipelines, the composition of the natural gas can change based on the native source of the LNG. As a result, it is beneficial to utilize analyzers that can detect changes in composition (and corresponding MN) in near real time. Optical technologies can offer both speed of response and compositional analysis. The MKS MultiGas™ Tunable Filter Spectrometer (TFS™) provides an alternative to the traditional analyzer technologies for hydrocarbon measurement, enabling low cost yet high performance analysis. The analyzer is permanently calibrated, reducing the need for costly reference gas mixtures and does not require fuel or carrier gases. The calibration stability is guaranteed through the use of a first-principle measurement technique and an advanced spectral processing algorithm that compensates for baseline variations. Automated calculation of MN can done in real time, allowing for real time protection and optimal control of IC engines.

Keywords: Fuels\Energy\Petrochemical, Gas, On-line, Process Monitoring
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Molecular Spectroscopy
Fuel theft in the form of diluting shipments using less expensive petroleum products continues to grow globally. Consequently, there exists a need to rapidly verify fuel quality during change of custody. Ideally such verification not only includes the fuel type, diesel, gasoline, or jet, but also their associated performance properties. Unfortunately, such verification must often be performed far from a lab containing standard fuel analysis instruments and apparatus. We have addressed this need by developing an inexpensive, compact (7x13x16", 14.5 lb) fuel analyzer based on near-infrared spectroscopy that employs multivariate statistics to determine numerous properties in less than 10 seconds using only 2 mL of sample. These properties include aromatic, olefinic, naphthene, oxygenate, and saturate content; cetane and octane numbers; distillation, flash, freezing, and pour points; density (API gravity), Reid vapor pressure, and viscosity. Here we describe the method to transfer the property models from a master analyzer to multiple slave analyzers in accordance to ASTM practices.

Keywords: Chemometrics, Fuels\Energy\Petrochemical, Method Development, Near Infrared
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Near Infrared
Determination of Benzo[a]pyrene and Benz[a]anthracene in Coal Tar and Pitch Products by Automated SPE-GC/MS

Abstract Text

This poster presents the automated determination of benzo[a]pyrene and benz[a]anthracene in tar distillation products by SPE-GC/MS. Samples are dissolved in tetrahydrofuran (THF), placed in sample vials and internal standard added. The vials are then placed in the autosampler. All further sample preparation steps which include SPE cleanup on silica gel cartridges and injection into the GC/MS are performed automatically. Eight replicates of each sample were analyzed resulting in relative standard deviations between 0.8 and 2.6%. Analysis results were compared with results of an LC reference method with fluorescence detection. Both methods gave equivalent results with maximum deviations of +6.7 and -11.3% for benzo[a]pyrene. These data show that the developed SPE-GC/MS analysis method is precise and accurate. The SPE system performs sample preparation during the chromatographic run of the preceding sample for maximum throughput, enabling the analysis of around 40 samples per day.

Keywords: Gas Chromatography/Mass Spectrometry, PAH, Sample Handling/Automation, Solid Phase Extraction
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Gas Chromatography/Mass Spectrometry
Pt-Sn/Al2O3 is the most important kind of catalysts in reform technology of refinery. The metals dispersion and the interaction between metal Pt and Sn and between metals and support play important roles in the performance and lifetime of catalysts. In this paper, a series of commercial and prepared catalysts were characterized by atomic resolution scanning transmission electron microscope (STEM) and CO probe in-situ FT-IR to study platinum dispersion on support and effect with additives metal tin. The spectral characteristics of probe molecule CO adsorbed on Pt active sites on catalyst surface can reflect the action of Sn metal from geometrical effect and electronic effect (see figure 1). The dispersion of Pt on the atomic scale of different catalysts can be observed directly by STEM image (see figure 2). The size and number of Pt atomic cluster decreased with the addition of metal tin. The lifetime and performance of Pt-Sn/Al2O3 catalyst compared with Pt/Al2O3 catalyst were significantly improved.

Keywords: Characterization, Microscopy, Molecular Spectroscopy
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Molecular Spectroscopy
Petroleum products are susceptible to deterioration when they are exposed to moisture in the environment. This results in corrosion on interior surfaces, damage in pipelines and form deposits in engines. The method used for the determination of the corrosiveness of gasoline and distillate fuels in preparation for transport through a pipeline was developed by National Association of Corrosion Engineers (NACE) named NACE TM0172 that takes 4 hours to perform with 300mL of sample. Due to high popularity of the test method, there has been a growing interest from the key stakeholders for a quicker method with faster turnaround, smaller sample size and better repeatability and reliability. Additionally, Silver corrosion is an important factor in automobile applications, and the test method for studying this process could also benefit from a quicker turnaround in the process as well.

In collaboration with key refineries around the country, and with numerous trial and error experimental setups, a quicker, easier, and more efficient method has finally been developed—the new ASTM test method, ASTM D7548 for Determination of Accelerated Iron Corrosion in Petroleum Products (AICT). The new test method only requires a 50-mL sample and less than a 1/4 of time to complete testing compared to the NACE TM0172 and ASTM D665. This accelerated method will be significantly efficient at pipeline transfer stations, where a quick QC turnaround time is key to determine the corrosiveness of products. Its revolutionary characteristics address some engineering concerns expressed by industry leaders regarding the accelerated corrosion test methods of the past. This study will discuss the development of this test technique, and the authors will also present results that show comparative data between the NACE test and ASTM D7548. Work that has begun on the Silver corrosion test will also be discussed.

Keywords: Fuels\Energy\Petrochemical, Gasoline, Method Development, Petroleum

Application Code: Fuels, Energy and Petrochemical

Methodology Code: Process Analytical Techniques
Bulky phenolic compounds, such as 2,6-di-tert-butyl-4-methylphenol, are well-known antioxidants with broad industrial, health and medicinal applications. Despite their wide uses, the structure-property-reactivity relationship of phenol-based antioxidants remains poorly understood. Our main objective is to use a multimodal approach combining electrochemistry, spectroscopy, mass spectrometry and high-performance liquid chromatography to gain new insight into reactivity of bulky phenols in a variety of radical reactions. This will lead to design of novel “super-antioxidant”. Cyclic voltammetry and square-wave voltammetry were used to monitor superoxide anion radical (SAR) reaction with bulky phenols. The relative reactivity of the phenolic compounds with SAR was highly dependent on the substitution pattern around a phenolic ring and number of substituents. The reactivity of phenol analogues with organic 2,2-diphenyl-1-picrylhydrazyl radical was measured by UV-vis spectroscopy and cyclic voltammetry. The phenolic compound reactivity in this assay was dependent on the degree of steric hindrance around the hydroxyl group and the electronic factor associated with the substituents. In addition, the oxidation of phenolic compounds was measured in a competitive and non-competitive assay with 3,3′-diaminobenzidine (DAB) in Horse Radish Peroxidase (HRP)-catalyzed reactions with hydrogen peroxide. BHT was preferentially oxidized over DAB compared to other phenol analogues, while some phenolic compounds selectively reacted with DAB intermediate to produce new coupling products. The peroxidation reactions were also characterized by ESI-MS and HPLC. Our results show that antioxidant structure of analogues dramatically modulates their reactivity. In addition, the relative reactivity order of these antioxidants was not conserved between assays.

Acknowledgment is made to the Donors of the American Chemical Society Petroleum Research Fund for support of this research.
Traditional leak detection processes do not meet today’s demands for fast, accurate and transparent data. Utilities, as well as gas transmission and distribution companies, face increased challenges with pipeline monitoring and compliance due to:
- Aging infrastructure
- Regulatory pressure to improve system integrity
- Desire to reduce greenhouse gas emissions
- Reliance on time consuming, error-prone paper-based monitoring systems
- Pressure to reduce cost
- Need for data transparency

The new natural gas leak detection system installed on a vehicle directly addresses all of these challenges using patented cavity enhanced laser-based technology. It’s inherent high sensitivity enables unambiguous identification of leaks several hundred feet away from the source. It has high reliability due to measure both methane and ethane to eliminate false positives. It saves time due begin finding leaks within 2 minutes after power on. Needs less maintenance which is unlike other laser methods, it is far more robust and can be fully serviced anytime, anywhere, by anyone. This leads to lower operating cost due customer owns the instrument and all data. It allows a transparent information handling due to data availability everywhere and immediately through cloud-based reporting. It consists of a Methane/Ethane analyzer, a GPS, a sonic anemometer and proprietary leak detection software that presents real-time geospatial maps of multiple gas concentrations in real time. The software’s sophisticated leak detection algorithm combines the system’s measurements of gas concentrations (CH4, C2H6), local coordinates (GPS) and local wind velocity (sonic anemometer) to estimate the location of the leak. This reduces the time necessary to pinpoint a leak, increasing safety and lowering emissions.

Readings are stored in the device and can be transmitted in real-time to the cloud for centralized monitoring. In the presentation some examples will be shown to highlight the new approach.

Keywords: Automation, Environmental Analysis, Environmental/Air, Gas
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Molecular Spectroscopy
Fuels, Energy, and Petrochemical Analysis

Oil Refinery Flare Gas Analysis and Its Impact on Environmental Regulatory Compliance

Vent gas streams from oil refinery process units are sent to a flare for destruction prior to release into the atmosphere. This practice is essential to the safe and responsible operation of the refinery, and has been the focus of several recent regulatory updates by the US Environmental Protection Agency. The intent of these changes is to reduce the release of hazardous air pollutants by controlling the composition of the gas sent to the flare. For example, an addition to the Refinery Sector Rule (RSR) requires the owner to maintain a Net Heating Value (NHV) over 270 BTU/scf in the combustion zone during any release of regulated material. This is intended to ensure efficient operation of the flare, and destruction of VOCs, but in order to remain above this limit, many sites will require active additions of supplemental gas, in the form of methane or propane, and steam. Compliance will depend on the refinery’s ability to monitor and react to changes within the vent gas composition. This discussion will review mass spectrometer data from oil refinery vent streams and focus on how specific compounds, like hydrogen, water, hydrocarbons, and hydrogen sulfide can impact the analysis, and affect strategies for effective flare control.

Keywords: Environmental Analysis, Fuels\Energy\Petrochemical, Mass Spectrometry, Process Control
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Process Analytical Techniques
The present contribution reports results from photoelectrochemical studies on Cu\textsubscript{2}O/WSe\textsubscript{x} photocathodes for solar hydrogen production in the presence of WSe\textsubscript{x} thin films as co-catalysts. Potentiostatic electrodeposition and thermal annealing were used for the in-situ preparation of robust Cu\textsubscript{2}O/WSe\textsubscript{x} films on fluorine-doped tin oxide glass (F:SnO\textsubscript{2} or FTO). The as-prepared films were characterized by scanning electron microscopy, energy dispersive X-ray analysis, and UV-vis spectroscopy. Under simulated solar irradiation, a maximum photocurrent of \( \sim 1.0 \text{ mA/cm}^2 \) was obtained from Cu\textsubscript{2}O/WSe\textsubscript{x} cathodes in 0.050 M pH 9 borate buffer electrolyte at -0.40 V vs Ag/AgCl (3.0 M KCl), which was a 8-times increase in photocurrent as compared to that from the cathode without using WSe\textsubscript{x}. The use of the catalyst also significantly shifted the onset potential of water reduction to a more positive value. The above increase in photocurrent and shift in onset potential could be attributed to electrocatalytic activity and the effect of catalyst on increasing electron-hole separation on the semiconductor electrolyte interface upon light illumination, as confirmed by electrochemical impedance spectroscopy. Although transition metal chalcogenides have been one of the key areas of research in the field of water oxidation and reduction catalysis and electrocatalysis, reports on their application as co-catalysts for solar water splitting have been relatively limited. Hence, investigations on the use of transition metal chalcogenide based materials as cost-efficient catalysts for water reduction could be crucial in constructing tandem cells for solar water splitting.
Bacterial fermentations are typically studied by focusing on: (i) secreted products accumulated in the culture media, or alternatively (ii) lysed cell membrane for internal contents. In this research, we seek to correlate intracellular cell metabolites data with extracellular outputs using the same analytical technique – Raman spectroscopy. *Clostridium acetobutylicum* cells were grown on glucose, gluconate and galacturonate as substrates in triplicate batch fermentations and sampled hourly through to the stationary phase. Samples were extracted for the following analysis: (i) optical density measurements at 600 nm – cell containing, (ii) extracellular metabolites – cell free (HPLC analysis) and cell containing (in-situ Raman optical probes), (iii) intracellular metabolites – washed cells (Raman microscopy). Statistical analysis will be employed to explore possible correlations within the datasets.

**Keywords:** Carbohydrates, Data Analysis, HPLC, Raman Spectroscopy

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

**Methodology Code:** Molecular Spectroscopy
The static water contact angle (CA) quantifies the degree of wetting that occurs when a surface encounters a liquid, e.g. water. This property is a result of factors such as surface chemistry, local roughness and is an important analytical parameter linked to the suitability of a surface for any bioanalytical process. Monitoring spatial variations in wettability over surfaces is increasingly critical to analysts and manufacturers. However, CA acquisition is often time consuming because it involves measurements over multiple spatial locations, independent sampling and the need for a single instrument operator. Furthermore, surfaces exposed to local environments specific to the intended application, may affect the surface chemistry, thereby modifying the surface properties. In this study, Attenuated Total Reflection – Fourier Transform Infrared (ATR-FTIR) chemical imaging data acquired from wet and dry polymer surfaces were used to develop multivariate predictive models for CA prediction. Partial Least Squares Regression (PLSR) models were built using IR spectra from surfaces presenting differences in experimentally measured CA (16° - 141°). A PLSR model built using wet spectra of surfaces performed better than a model built using native dry spectra in terms of prediction model indicators when tested on an independent experimental set. The optimal models were subsequently applied to IR spectra acquired from a surface exhibiting spatial differences in surface chemistry and CA, demonstrating the potential of this method for prediction of spatially varying CA.

Acknowledgement:
The authors gratefully acknowledge funding from the EU FP7 under the European Research Council Starting Grant programme (ERC-SG-335508).

Keywords: Imaging, Materials Characterization, Surface Analysis, Vibrational Spectroscopy
Application Code: Material Science
Methodology Code: Surface Analysis/Imaging
In recent years, there has been an increasing interest for the direct analysis of sample substrate surfaces with atmospheric pressure plasma jet (APPJ) sources. Depth profiling of sample substrates via ambient desorption/ionization mass spectrometry has been proposed by using an APPJ micro-capillary dielectric barrier discharge (DBD). Nevertheless, the underlying mechanisms of how the discharge interacts with the sample substrate surface during the erosion process are far from being completely understood. Herein, optical emission hyperspectral images of a Helium–DBD (30kHz AC), covering the region where the discharge effluent interacts with the sample surface in the open air, will be presented. A Fourier-based Abel inversion algorithm will also be implemented to reconstruct the radial distribution of the plasma optical emission. The capillary DBD plasma has a miniaturized configuration of 5 cm long, 360 μm o.d. and 150 μm i.d., and the limited number of spatial data points make noise suppression protocols particularly important. The Fourier-based Abel inversion, as opposed to typical Abel inversion algorithms, has the advantage of avoiding noise accumulation from the plasma edge region toward the axis. Hyperspectral images obtained under different plasma operating conditions, such as helium flow rate, applied voltage, and sample substrate will be compared. Preliminary results reveal emission at 639.9 nm and 513.3 nm which correspond to helium excimer(He2). Time-resolved spectra along the period of the current waveform will also be obtained. The results will help to obtain a better understanding of the energy transfer in the DBD and its interaction with the sample surface during erosion.

Keywords: Method Development, Plasma Emission (ICP/MIP/DCP/etc.), Spectroscopy, Surface Analysis
Application Code: General Interest
Methodology Code: Surface Analysis/Imaging
Silver-EDTA Nanoparticle Decorated PVA Nanofibers for Reversible Capture and Quantification of Proteins

Functional surface to capture proteins has great biological implication and has been studied broadly using antibodies. However, antibodies are costly, have orientation issues, prone to both cross-talking and non-specific binding. An alternative to antibodies are metal ion affinity binding, as used in NTA-Nickel columns for HIS-tagged protein extraction. In this work, we report the fabrication of electrospun polyvinyl alcohol (PVA) nanofibers treated glutaraldehyde (GA) cross-linking to resist degradation in an aqueous environment; these nanofibers are functionalized with ethylenediaminetetraacetic acid (EDTA) capped silver nanoparticles which provide an effective surface for metal ion chelation. Additionally, the layer of silver nanoparticles improves the optical properties of the fiber mat, providing local surface plasmon resonance (LSPR) from these silver nanoparticles. Metal ions such as Silver(I), Copper(II), and Nickel(II) were studied for its binding to the surface and as well as the LSPR response of the fiber surface to the binding of these molecules. These chelated metal ions, Nickel(II) on the surface can be utilized as bridge between the surface to marked proteins such as HIS-tagged and NTA-tagged compounds can be bound and sequestered on the surface for the detection and quantification of these tagged molecules on the substrate. A fluorophore, Rhodamine, was added to the tagged proteins to enable quantification of the amount of proteins captured by this novel surface.

Keywords: Bioanalytical, Biosensors, Fluorescence, Spectroscopy
Application Code: Bioanalytical
Methodology Code: Surface Analysis/Imaging
Cellulose nanofiber is the most advanced biomass material which is generated by defibrating to the level of nanometers in length cellulose which is a main component of cell walls and plant cells. The forming technique development of cellulose nanofiber requires the evaluations such as size, cohesiveness and transparency. An atomic force microscope (AFM) which is an indispensable analytical technique for the evaluation of the size and cohesiveness has a complicated analysis process imposing a burden for an analyst. In order to resolve the issue, we examined the use of a nano particle size analyzer and a UV-Visible spectrophotometer which were easier and quicker than an AFM. Water-dispersive cellulose and carboxymethyl cellulose (CMC) which was a cellulose derivative with carboxymethyl groups (-CH2-COOH) bound to some of the hydroxyl groups of the glucopyranose monomers which made up the cellulose backbone were measured with a UV-Visible spectrophotometer, a nano particle size analyzer and an AFM to investigate their transparency, microscopic structure, cohesiveness and size. The result for the size obtained with a nano particle size analyzer showed the good correlation with that for an AFM. A nano particle size analyzer also enabled the quantitation of cohesiveness. The transparency measured with a UV-Visible spectrophotometer showed the interesting correlation with the length and dispersibility of fibers obtained with an AFM. In this work we demonstrate their spectroscopic and structural study. The following figures are shown to understand the abstract written above.

Keywords: Analysis, Environmental, Material Science, Nanotechnology
Application Code: Material Science
Methodology Code: Surface Analysis/Imaging
Surface Analysis and Imaging

Real Time Viability Evaluation and Monitoring of Rat Cardiomyocyte Using Surface Plasmon Resonance

Being the leading cause of death globally, Cardiovascular Diseases (CVD) vary from different types of strokes, cardiomyopathies, hypertension, and heart failure. Animal and human trials are the foremost option for drug testing, disease modeling, and biomarker analysis. These approaches have the benefit of providing a systematic view rather than just assessing the heart, however, they require significant labor, time, and cost. Microfluidics and lab-on-chip devices are fast emerging in the recent years. They are meant to decrease the need for clinical trials and fast forwarding the drug marketing development. However, there still lies a need for better optochemical technologies to detect desired biomarkers and study CVD models in their original physiological condition. Surface Plasmon Resonance (SPR) technology employs an incident laser light and its refractive angle to respectively stimulate and read from resonant oscillation of conduction electrons at the interface between negative and positive permittivity material. In this research, we report a novel live-cell SPR platform to measure and characterize the contractibility of beating cardiomyocytes. Rat cardiomyocytes were isolated from neonatal rat hearts day 1 through 3 and cultured on SPR gold chips. Their spontaneous contraction under normal and drug induced conditions were monitored using SPR technology. Our research provides not only live monitoring of beating characteristic for cardiomyocytes, but also a comprehensive kinetic analysis of their released biomarkers using detection-specific designed gold chips.

Acknowledgement: This work was supported by the Engineering Research Centers Program of the National Science Foundation under NSF Cooperative Agreement No. EEC-1647837.

Keywords: Biosensors, Drugs, Magnetic Resonance, Surface Analysis
Application Code: Biomedical
Methodology Code: Surface Analysis/Imaging
Ionic liquids (ILs) are salts with low melting points. By the virtue of their structural variety and unique properties such as low vapor pressure, thermal stability and conductivity, ILs are used in several applications e.g., lubrication, energy storage devices, catalysis, and biomass solvation. Most of these applications rely on the chemical and physical interactions of the solid-liquid interface. Liquid molecules are known to self-assemble themselves into ordered structures at the solid surface to form an interfacial region which displays properties different from bulk liquid. ILs are known to form interfacial films that extend up to tens of nanometers from a solid surface.1-3 The hygroscopic nature of ILs renders water as the most common impurity in them. The presence of water can have major impact on their behavior. Hence, understanding influence of water on the interfacial structure of ILs becomes very important. Our research employs a dynamic wetting technique to create and allow direct spectroscopic probing of IL films on solid substrates. Using IRRAS, sum frequency generation, and ellipsometry we correlate changes in the vibrational spectra of IL films to their molecular orientations and intermolecular interactions. Our results describe reversible, long-range ordering in IL films that extends 0.8 nm to 2 nm from the solid substrate into the liquid phase. This is ca. 10 times farther than the ordering that has been reported in previous studies. Effect of water on these IL films as a function of substrate rotational velocity and time are compared to understand water-IL interactions.


Keywords: Analysis, Spectroscopy, Surface Analysis, Vibrational Spectroscopy
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Surface Analysis/Imaging
Porphyrin molecules are highly conjugated macrocyclic organic molecules. Due to their unique electronic structure these molecules can function as photocatalysts, aiding in the break-down of air pollutants. Further, porphyrins such as tetrakis(4-carboxyphenyl) (TCPP) have been shown to self-assemble into nanostructures with enhanced photocatalytic properties compared to their monomer form. One example is TCPP nanowires, formed through titration with hydrochloric acid (HCl). The nanowires form through electrostatic interactions of the protonated TCPP core and chloride ions, thus the nanostructures can be controlled under different pH conditions. Nanowires are formed at pH values below 2, but nanoplatelets are observed at a pH of 3. The mechanism driving the transition between assembled TCPP nanostructures, however, is not known. Therefore, the goal of this work is to explore the structural details of TCPP assemblies under systematically changed pH conditions. Atomic Force Microscopy (AFM) was utilized to monitor the morphological changes of TCPP nanostructures. The nanowires measured 0.6-1.5 μm in length with diameters of 100-300 nm. Both global and local changes in nanowire structure were measured by either immersing the entire sample in specific pH solutions for set times or using Dip-pen nanolithography. For Dip-pen nanolithography, the AFM tip was used to induce local structural changes utilizing humidity to alter the pH where the AFM tip is in contact with the sample. Preliminary results suggest that in pH 3 solution the wires disassemble primarily in the longitudinal direction at a growth rate of 4 nm/min. When placed in pH 1 the wires assemble at 9 nm/min.

Keywords: Atomic Force Microscopy (AFM), Materials Characterization
Application Code: Material Science
Methodology Code: Surface Analysis/Imaging
Towards the development of Surface Plasmon Resonance imaging (SPRi) chips for a panel of microRNA strands we explored the effect on the probe immobilization on both the hybridization efficiency and kinetic parameters of the hybridization assays. We found that microspotting of terminal thiol labelled DNA onto gold needs careful optimization to overcome problems related with the nonspecific surface adsorption of DNA strands as well as to establish optimal surface densities for subsequent hybridization with microRNAs [1]. While most optimization of end point based hybridization assays are considering the hybridization efficiency (maximizing the amount of complementary strands bound) as dominant criteria, here, by taking advantage of the kinetic information provided in high throughput manner by SPRi we are able report comprehensive data on the optimal surface densities for kinetically controlled selectivity to discriminate mismatched strands as well as for the unbiased $K_{D}$ determination. For accurate kinetic measurements we propose microspotting of thiol labeled DNA probes both in prehybridized form with a complementary DNA (phDNA) as opposed to the conventionally used single-stranded DNA (ssDNA). We found that phDNA self-regulates the optimal surface density of DNA probes at sufficiently high spotting concentrations after removal of the prehybridized complementary strands, i.e., activation of the DNA probes. We correlated these reagentless investigations with SPRi based determination of the probe density using hexaammineruthenium(III)-solution [2].

Figure 1.— SPR image of DNA spots immobilized in different concentration and form during injection of A.a) microRNA and A.b) hexaammineruthenium(III). B) Dissociation constants of DNA – microRNA hybridization.

References

Keywords: Biosensors, Immobilization, Method Development, Optimization
Application Code: Bioanalytical
Methodology Code: Sensors
Ion-selective electrodes (ISEs) are widely used for selective measurement of ion activities in clinical and environmental analysis\[^{1}\]. Most often these electrodes take advantage of the excellent selectivity of a highly lipophilic ionophore that is incorporated in hydrophobic plasticized polymer membrane. However there is a large range of natural ligands with excellent ion-selectivities, but the hydrophobic membrane matrix impedes the use of such hydrophilic complexing agents. We propose a new membrane construction that is based on the use of gold nanoporous membranes to which thiol functionalized compounds can be linked. We have shown earlier that such nanoporous membranes can form the basis of silver ISEs with excellent selectivities\[^{2}\]. Here we show that the concept can be extended to hydrophilic ligands as demonstrated by using different peptides with different amino acid sequences, each of them known to bind a heavy metal ion (e.g. Cu\[^{2+}\], Cd\[^{2+}\], Pb\[^{2+}\]) with considerable selectivity. For Cu\[^{2+}\] ions the potentiometric investigation revealed Nernstian response for in the 10\(^{-3}\) to 10\(^{-5}\) M region, with limit of detection of ca. 10\(^{-7}\) M. The selectivity of the nanoporous membrane based Cu\[^{2+}\] ISEs matched that of the state of the art ionophore-based ion selective electrodes for the most relevant cations. Moreover, as all active components are covalently bound to the nanoporous support, the proposed peptide-based nanopores are exhibit longer lifetime than the conventional plasticized polymeric membrane based ISEs, from which the active components continuously leach during use.

Acknowledgment
This work was supported by the Lendület program of the Hungarian Academy of Sciences (LP2013-63).

References

Keywords: Environmental Analysis, Method Development, Potentiometry, Sensors
Application Code: Environmental
Methodology Code: Sensors
The success rate in obtaining usable latent fingerprints from metal surfaces, including gun shell casings, is extremely low, so new methods are needed. Sebaceous fingerprint residue insulates a portion of the surface, allowing electrochemical polymerization processes to occur in the valleys of the fingerprint pattern while leaving the peaks uncoated. Electrochemical oxidation of heterolenes (thiophene, 3-methyl thiophene, 3,4-ethylenedioxythiophene (EDOT), pyrrole and aniline) produced intensely dark conducting polymer films of good contrast. Comparison of polymerization in organic and ionic liquid solvents showed better contrast in ionic liquid systems, presumable since the more polar ionic liquids dissolved less of the sebaceous fingerprint residue. EDOT concentrations above 100 mM in 1-butyl-3-methylimidazolium tetrafluoroborate (BMIBF4) produced optimal coatings. Controlled current studies with charge densities in the range of 275-375 coulombs cm\(^{-2}\) applied over a period of 20 seconds resulted in dark polymer coatings 230-300 nm thick which were judged suitable for identification according to the Bandy scale. Chronoamperometry studies allowed design of a portable battery-operated two electrode system. Fingerprints on irregular shaped objects (doorknobs, spoons, coffee mugs) were enhanced using cells made with ethyl vinyl acetate foam and a stainless steel plate counter electrode. Photography of the print on irregular metal objects could be difficult, so various tapes were evaluated to identify if they could lift the polymer coating. In an evaluation of 12 brands of tape, D-Squame (CuDerm Corp., Dallas, TX) performed the best.

**Keywords:** Electrochemistry, Forensics, Paint/Coatings, Portable Instruments

**Application Code:** Homeland Security/Forensics

**Methodology Code:** Electrochemistry
Ion-selective electrodes (ISEs) are potentiometric sensors for the selective determination of target ions in samples. Measurement requires not only ISEs for target ions but also a reference electrode that generates constant electric potential. A reference electrode and sample solution should be connected with reference solution (in other word, bridge electrolyte) which consists of two ions with similar mobility in order to reduce liquid junction potential between sample solution and reference solution. Although combination of a silver/silver chloride reference electrode and KCl solution is the most general choice, considering the low concentration of K ion in blood, lithium acetate (LiAcO) solution would be better reference solution for blood-ion determination especially for a flow-type electrolyte cartridge which has limited space to avoid contamination of reference solution into sample solution.

In this study, we combine a lithium-selective ISE as a reference electrode and LiAcO aqueous solution as reference solution to demonstrate the lithium-selective reference electrodes for flow-type electrolyte cartridges. The cartridge has Na, K and Cl-ISEs located on left-hand side of a flow channel (1 mm width and 30 mm length) and Li-ISE located on right-hand side. The flow channel was filled with sample solution and LiAcO solution as reference solution and two solutions formed liquid junction on the center of flow channel. The cartridge showed good slopes (>58 mV/decade) against Na, K and Cl ions. Regarding the potential stability, fluctuation was less than 0.25 mV in 3 minutes which corresponds to 1% error of ion-concentration determination.

Keywords: Electrochemistry, Electrodes
Application Code: Bioanalytical
Methodology Code: Electrochemistry
Electrophoretic Immobilization of Bacteria with Carbon Nanotubes to Study Bacterial Metabolism Using Scanning Electrochemical Microscopy (SECM)

Primary Author: Joseph Kincaid  
Oregon State University

Co-Authors: Dipankar Koley

Abstract Text:

[i]Streptococcus mutans[/i] bacteria were electrophoretically co-deposited with functionalized multiwalled carbon nanotubes (f-MWCNTs) on the surface of a platinum ultramicroelectrode (UME), thereby immobilizing the bacteria in a CNT matrix and preventing it from separating from the electrode and moving into the surrounding solution. The proportion of bacteria in the matrix is being controlled by adjusting the CNT/bacteria ratio and deposition time. We will use a dual electrode as a probe in scanning electrochemical microscopy (SECM); one electrode with bare Pt to fix the distance using a negative approach curve and the other to deposit [i]S. mutans[/i]/CNT. This unique set up will enable us to study metabolic interactions between two bacterial species at various distances. This will be accomplished by using an SECM with a solid-state pH sensor which gives Nernstian slope (58±2 mV/pH) as the substrate electrode and the CNT/bacteria-deposited Pt UME as the working electrode.

Keywords: Bioanalytical, Electrophoresis, Potentiometry
Application Code: Bioanalytical
Methodology Code: Electrochemistry
We have developed a solid-state potentiometric pH wire sensor that can be used to detect bacterial metabolites at the biofilm-substrate interface in real-time. The sensor is composed of a PVC based membrane with carbon nanotubes as a back contact on a 100 µm diameter Pt wire. The Pt wire base allows for the sensor to be highly flexible allowing for easy positioning of the sensor tip. The sensors showed near-Nernstian responses, with slopes of 48±5 mV, with very fast response times of 1 second. The sensors were later attached on the biomaterials and [i]Streptococcus mutans[/i] biofilm was grown on the Ca[sup]2+[/sup] ion releasing biomaterials. The latest findings of the real-time change in pH and Ca[sup]2+[/sup] at the interface will be presented at the meeting.

**Keywords:** Bioanalytical, Biosensors, Ion Selective Electrodes, Potentiometry

**Application Code:** Bioanalytical

**Methodology Code:** Electrochemistry
Metastable-state photoacids (mPAHs) are an emerging family of photochromic compounds with attractive characteristics. Unlike UV light-activated spiropyrans, mPAHs have the advantage of photoisomerizing with visible light(1). Also, the reversible behavior of mPAHs allows for pH control over several cycles, in contrast with single-use photoacid generators. These properties prompted the use mPAHs as reaction catalysts(2), ion-channel activators(3), and novel chromoionophores in ion-selective optodes(4).

In order to better understand these compounds we perform Density Functional Theory (DFT) calculations on different mPAH derivatives. The results of these calculations allow to predict experimental spectra, visualize electron distribution, and model molecular orbitals. By comparing with experimental data, we are able to find the best theoretical model to describe mPAHs. Using this model, we determine the effect of substituents on the different properties of the molecule and elucidates the photoisomerization process.

This study establishes DFT calculations as a method to predict the absorbance spectrum of mPAHs. As a result, DFT modeling can be used to narrow the synthesis options of these compounds to candidates with desired optical properties such as a particular absorbance profile. Additionally, the data garnered from this project will serve as a base for preliminary calculations on the next generation of mPAHs. By shedding some light into the nature of these interesting molecules, this study aims to foster the use of mPAHs for application in the areas of bio-imaging and smart sensors.

Micro-ion selective electrodes (-ISEs) are a reliable, sensitive and selective tool for detecting ionic concentrations in a variety of matrices [1]. Monitoring ionic deficiencies within the phloem and xylem bundles of citrus plants are particularly of interest due to citrus greening disease (CGD), which has been linked to zinc deficiency in plants. Therefore, a tool is required to monitor varying concentrations of zinc in plants to assess disease progression and possible therapy [2]. Well established tools for plant analysis such as Atomic Absorption Spectroscopy (AAS), X-Ray Fluorescence, among other methods are costly, non-portable and destructive [3]. Therefore, here solid-contact -ISEs will provide a non-destructive alternative method for monitoring free zinc content in the phloem of plants, via a small incision. Here, a µ-solid-contact ISE with a 600 µm diameter was built by coating a gold wire with a conducting polymer (poly-octylthiophene), providing good reproducibility among electrodes and promising LODs. The sensors were developed using a cocktail mixture of Zinc Ionophore I (80 mmol/kg) to KTFPB (10 mmol/kg) with a plasticized PVC matrix. Studies yielded a near Nernstian slope of 27.9 ± 1.09 mV with a limit of detection (LOD) of 8.53 ± 0.07 x 10^-7 M within 10^-7 to 10^-2 M range. Analysis is under optimization in plant matter with promising responses. It is expected to create an all solid-contact micro-ISE platform for plant analysis.

References
(1) Solsky, R. L. Analytical Chemistry 1990, No. 62, 21R–33R.

Keywords: Electrochemistry, Environmental Analysis, Ion Selective Electrodes, Microelectrode
Application Code: Environmental
Methodology Code: Sensors
Severe sodium ion (Na$^{+}$) discrepancies caused by fluctuation of water level within blood, leads to hyponatraemia and hypernatremia.\[1\] As a consequence, heart failures are often associated with the more severe cases, resulting in 17 million deaths per year worldwide.\[1,2\] Therefore, an on-demand, cost-effective point-of-care Na$^{+}$ monitoring is of great importance. Herein, we propose a novel approach to monitor Na$^{+}$ with high sensitivity, selectivity and reproducibility by utilizing a visible-light activated Na$^{+}$-selective optode in microfluidic device. The sensing membrane contains a fluorophore, a metastable-state photoacid (which provides reference ion after visible-light activation for ion-exchange process) and sodium ionophore X (selective towards Na$^{+}$) within a plasticized polymer matrix.\[3\] The microfluidic device was fabricated as a straight channel using multi-layered plastic of cyclic olefin polymer and pressure-sensitive adhesive.\[4\] The middle part of the channel contains a chamber for placing the sensing membrane. The resulting microfluidic sensor was tested with 200 µL of Na$^{+}$ standard solutions (from 1.0x10^{-7} to 1 M) at different pH. Based on absorbance and fluorescence analysis, the response time is shortened to few minutes at physiological parameters, while sensitivity and reproducibility for fluorescence measurements were drastically improved. Additionally, this sensor displays high tolerance towards other physiological ions (potassium, calcium and magnesium).

\[1\] R. M. Reynolds, P. L. Padfield, and J. R. Seckl, \[i\]BMJ[/i], \[b\]2006[/b], \[i\]332[/i], 702-705.
\[2\] http://www.world-heart-federation.org
\[3\] P. K. Patel, and K. Y. Chumbimuni-Torres, \[i\]Analyst[/i], \[b\]2016[/b], \[i\]141[/i], 85-89.
\[4\] J. Saez, L. Basabe-Desmonts, and F. Benito-Lopez, \[i\]Microfluid Nanofluid[/i], \[b\]2016[/b], \[i\]20[/i], 116.

Keywords: Fluorescence, Lab-on-a-Chip/Microfluidics, Sensors, UV-VIS Absorbance/Luminescence
Application Code: General Interest
Methodology Code: Sensors
A Selective Electrochemical Sensor for the Detection of the Zika Virus

With the rising medical concern towards the Zika virus, a four-way junction (4WJ) electrochemical sensor was developed to detect sequences of the virus for point-of-care (POC) analysis. The 4WJ sensor consists of a DNA stem-loop (SL) probe immobilized to a gold electrode surface and two intermediate adaptor strands. Both adaptor strands have one segment complementary to the SL probe and another segment complementary to the target. One of the adaptor strands is labeled with a methylene blue (MeB) redox marker. In the presence of the target, the SL opens to bind to the adaptor strands which in turn hybridize to the target sequence to form a 4WJ structure. The redox of MeB can be monitored using square wave voltammetry (SWV) upon formation of the 4WJ. The length and concentration of the SL probe was optimized to obtain the maximum signal upon target binding. The results indicate that a 0.1 \( \mu \text{M} \) SL solution with one thymine base between the hairpin loop and the thiol bond was optimal. The performance of the sensor was investigated using synthetic target fragments of the Zika virus from Uganda (KX421193.1) of varied length (33, 84, and 141 bases). The limit of detection (LOD) was determined to be 1 nM. The sensor presented here has shown great promise for rapid POC testing for the Zika Virus.

Keywords: Electrochemistry, Electrode Surfaces, Immobilization, Sensors
Application Code: Bioanalytical
Methodology Code: Electrochemistry
Traditional neurotransmitters and neuromodulators, like catecholamines and indolamines, can be released from immune cells to aid in inflammatory modulation. Melatonin, an electroactive neurotransmitter released from the pineal gland, is involved in maintaining a circadian rhythm and exhibits immunomodulatory effects within the immune system, particularly during inflammatory disease. Lymphocytes, like T-cells, express receptors and synthesis machinery for many neurotransmitters like melatonin; however, the extent to which these classic central nervous system analytes are released and cleared within the immune system is not fully understood. Furthermore, monitoring rapid release within the immune system has been difficult with existing analytical methods in immunology. Fast-scan cyclic voltammetry (FSCV) coupled to carbon-fiber microelectrodes is an electrochemical technique widely used to study neurotransmitter release in the brain and provides the necessary temporal resolution needed to monitor rapid changes. To date, the electrochemical behavior of melatonin has not been fully characterized at carbon-fiber microelectrodes with FSCV. Here, melatonin detection was optimized using FSCV by fine-tuning the electrochemical waveform for optimal detection among interferences, determined the limit of detection and dynamic range, and examined the stability of melatonin detection over time. As proof of principal, rapid changes in melatonin concentrations in slices of lymphoid tissue was detected. This provides the first optimized method of rapid melatonin monitoring in the immune system which will allow real-time monitoring of melatonin regulation of inflammation.
**Abstract Title**

**Improved Reference Electrodes for Electrochemical Measurements**

**Primary Author**

Evan L. Anderson  
University of Minnesota

**Co-Author(s)**

Marc A. Hillmyer, Philippe Buhlmann, Stacey Saba, Sujay Chopade, Timothy P. Lodge

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**Abstract Text**

Reference electrodes are an essential part of almost every electrochemical measurement. Ideally, they allow for reproducible and invariant potential differences across the reference electrode when immersed into sample solutions of different ionic strengths and compositions. Commercially available reference electrodes attempt to approach this ideal by using a porous glass that is filled with an electrolyte solution. This porous junction allows for electrical contact between an inner filling solution and sample solutions while preventing mixing. Unfortunately, the surfaces of these porous glass materials are electrically charged, resulting in sample-dependent electrostatic charge–charge interactions at the pore surfaces. These interactions lead to changes in the reference potential with sample compositions of up to 150 mV.[superscript 1] To overcome this, two types of reference electrodes, made with nanoporous polymeric materials or ionic liquid filled nanopores, were designed and evaluated. Both types of reference electrodes have greatly improved reference potentials and stabilities in solutions of varying ionic strengths and composition. Moreover, the ionic liquid based reference electrodes show only two percent of the variation in reference potential that commercial electrodes exhibit.[superscript 2]

2. [i]ACS Sens.[/i] [b]2017[/b], [i]2[/i], 1498-1504.

**Keywords:**  
Electrochemistry, Electrodes, Environmental/Water, Potentiometry

**Application Code:**  
Environmental

**Methodology Code:**  
Electrochemistry
Reference Electrodes with a stable potential are a vital component in developing electrochemical sensors. Ionic liquid (IL) junction based reference electrodes offer unique advantages compared to the conventional free flow double junction reference electrodes. Previously developed IL based polymeric reference electrodes require a plasticizer that leaks out gradually. This may cause inflammatory reaction when used as implanted sensors. Here we aim to overcome these drawbacks by expanding the IL based reference electrodes to biocompatible materials that do not require a plasticizer, such as silicone. In this work, we tested 7 different commercially available silicone materials. Most of them are compatible of solvent casting and drop casting fabrication methods. Reference electrodes with Dow Corning 730 fluorosilicone as matrix show very stable potential in KCl range from 1 mM to 16 mM which covers the physiological K\(^{+}\) range. Excellent potential stability is also obtained in blood electrolyte solutions representing the most extreme conditions expected for implantable sensors. In long-term stability test, reference electrodes based on Dow 730 fluorosilicone give a minimal potential drift of 20.2 µV/h in 8 days. Differential scanning calorimetry tests further reveal the difference in miscibility between the IL and different polymeric materials. Good miscibility between IL and polymer leads to functional reference electrode, whereas poor miscibility is indicative of unstable potentials. The development and understanding of biocompatible material based reference electrodes pave the way for fully implantable sensors that are vital in clinical diagnostics and health monitoring.
The electrode-to-electrode reproducibility of current solid-contact ion-selective electrodes (ISEs) is unsatisfactory due to the ill-defined interfacial potential between the ion-selective membrane and the underlying conductor. Therefore, each electrode needs to be calibrated frequently, which is inconvenient for routine analysis and impedes electrode implantation into the human body. In this work, a cobalt(II/III) polymeric redox buffer was developed by covalently attaching cobalt(II/III) bipyridyl complexes to methacrylate copolymer chains. It served as an inner reference for solid-contact ISEs, and the electrode-to-electrode reproducibility was improved significantly compared to coated-wire solid-contact ISEs. The redox buffer was compatible with ionophore-doped solid-contact ISEs, eliminating leaching from the sensing membrane into the sample.


Keywords: Analysis, Electrochemistry, Electrodes
Application Code: Clinical/Toxicology
Methodology Code: Electrochemistry
Carbohydrate metabolism of human oral microbes plays a key role in their survival inside the oral cavity and causing infectious diseases by conditional pathogenic microbes. Some species like [i] Streptococcus mutans (Sm) [/i] (species responsible for forming dental cavities), consumes glucose or sucrose and produce lactic acid as the byproduct. While these byproducts decrease the pH of the saliva within those bacterial micro habitat, microbes like [i] Aggregatibactor actinomycetemcomitans (Aa) [/i] (which cause periodontitis) can catabolize lactate as their carbon source. Even though there is a high demand, very limited real time quantitative data is available about this carbohydrate metabolism.

Scanning electrochemical microscopy (SECM) can be used to obtain real time quantitative data of small molecules at a submicron distance from a surface of interest. Here we have developed, SECM compatible, highly sensitive, enzyme based ultra-micro lactate and glucose sensors which can quantify respective analytes above a microbial biofilm. Further, developed sensors will be combined with an ultra-micro pH sensor for accurate quantification of the lactate concentration profile above the biofilms. Fabricated sensors will be used with SECM to obtain real time quantitative data about carbohydrate metabolism of oral microbial biofilms.

**Abstract Text**

Carbohydrate metabolism of human oral microbes plays a key role in their survival inside the oral cavity and causing infectious diseases by conditional pathogenic microbes. Some species like [i] Streptococcus mutans (Sm) [/i] (species responsible for forming dental cavities), consumes glucose or sucrose and produce lactic acid as the byproduct. While these byproducts decrease the pH of the saliva within those bacterial micro habitat, microbes like [i] Aggregatibactor actinomycetemcomitans (Aa) [/i] (which cause periodontitis) can catabolize lactate as their carbon source. Even though there is a high demand, very limited real time quantitative data is available about this carbohydrate metabolism.

Scanning electrochemical microscopy (SECM) can be used to obtain real time quantitative data of small molecules at a submicron distance from a surface of interest. Here we have developed, SECM compatible, highly sensitive, enzyme based ultra-micro lactate and glucose sensors which can quantify respective analytes above a microbial biofilm. Further, developed sensors will be combined with an ultra-micro pH sensor for accurate quantification of the lactate concentration profile above the biofilms. Fabricated sensors will be used with SECM to obtain real time quantitative data about carbohydrate metabolism of oral microbial biofilms.

**Keywords:** Biosensors, Carbohydrates, Electrochemistry, Microelectrode

**Application Code:** Bioanalytical

**Methodology Code:** Electrochemistry
We examined the feasibility of fabricating plasticizer-free ion-selective optodes on non-porous transparent plastic sheets. Optical ion sensing ingredients including a pH indicator (chromoionophore), an ion receptor (ionophore), and a counterion (ion-exchanger) are deposited onto various transparent plastic sheets via either inkjet printing or manual coating techniques. It was found that selective colorimetric response toward a target ion such as Na$^+$ could be obtained as long as the sensing chemicals stay on the surface of plastic. Unmodified polyethylene terephthalate (PET) sheet and Highland® transparency film (with confidential proprietary surface) are excellent candidates of the transparent flexible substrates suitable for preparing such optodes. However, no color change could be observed once the sensing chemicals are dissolved into the plastic. Indeed, sensing chemicals prepared in cyclohexanone or tetrahydrofuran could be dissolved into clear polycarbonate and polystyrene sheets because of the poor solvent compatibility of these plastics, but not the PET and Highland sheets. Compared to our previously reported plasticizer-free paper-based ion-selective optodes, the transparent plastic sheet-based optode enables color recording from the backside of the optode (the side without the sensing layer) and circumvents mechanical property loss caused by substrate swelling. Therefore, this technology is well-suited for continuous wearable sensing applications in a cost-effective fashion. Moreover, the transparent substrate-based optode is compatible with spectrophotometric and fluorimetric detection apart from the camera-based colorimetric detection conventionally used for paper-based optodes.


Keywords: Bioanalytical, Clinical Chemistry, Polymers & Plastics, Sensors
Application Code: Biomedical
Methodology Code: Sensors
Potentiometric sensing of ions with ion-selective electrodes (ISEs) is a technique that is commonly used for selective and sensitive measurement of ions in complex matrices. Unfortunately, most commercially available ISEs and reference electrodes are bulky, delicate, expensive, and are not suitable for affordable, in-field, or point-of-care measurements. In this work, thread is used as a substrate for fabrication of robust and miniaturized solid-contact ISEs. Thread is inexpensive, widely available in a variety of materials (e.g., Nylon, cotton, polyester, wool), light, flexible, mechanically strong (both in dry and wet state), and comes in dimensions of smaller than 100s of microns (does not require patterning to define channel sizes).

Threads were coated with conductive inks and the ion-selective and reference membranes to develop thread-based reference electrode and ISEs selective to Cl⁻, K⁺, Na⁺, and Ca²⁺. Different types of thread (Nylon, cotton, polyester, and polypropylene) and a variety of conductive ion-to-electron transducer inks (carbon graphite, carbon black, and conductive polymer based on poly(3,4-ethylenedioxythiophene)) provided thread-based ISEs with Nernstian responses. The thread-based ISEs have broadly adaptable multiplexing capabilities. ISEs selective for different ions can be bundled with the reference electrode to allow multiplexed ion-sensing in small volumes of sample. The ISE bundle can easily be disassembled, and the individual ISEs and reference electrode can be used again to fabricate a different ISE bundle (for sensing of a different set of ions). The application of the thread-based ISE cell for detection of physiological electrolytes in human serum and urine is demonstrated.

Keywords: Biosensors, Clinical Chemistry, Electrochemistry, Ion Selective Electrodes
Application Code: Clinical/Toxicology
Methodology Code: Sensors
New methods of detecting biomarkers of liver functionality is an important field of research with ramifications in both analytical chemistry and the health industry. From an analytical chemistry point of view, the fabrication of new devices with new applications often results in the advancement of the field in general. Unfortunately, the transition of new science to actual implementation in relevant health industries routinely fails. One way to overcome this hurdle is to develop point-of-care (PoC) devices for biologically relevant and medicinally important markers of health. A current major concern in the United States is the opioid epidemic, and a common effect of opioid drug use, especially when taken in conjunction with other medications, is liver disease. In fact, deaths have more than tripled since 1999 with over 16000 fatalities attributed to prescription opioids in 2013 alone, making the development of PoC devices capable of detecting liver biomarkers an important endeavor. This work deals with the fabrication and development of an electrochemical sensor for the sensitive and selective detection of liver biomarkers at the point-of-care. A conventional approach to the design of the sensor was undertaken and optimized before the transition to a paper-based PoC device. The developed sensor can quantify specific analytes over normal physiological levels as well as covering the elevated concentrations common in individuals whose liver function is in peril.

Keywords: Electrochemistry, Lab-on-a-Chip/Microfluidics, Sensors
Application Code: Bioanalytical
Methodology Code: Electrochemistry
Potentiometry is a technique that is commonly used for sensitive and selective detection of ions in complex matrices. The experimental setup for potentiometric measurements consists of an ion-selective electrode (ISE) and a reference electrode, immersed in the test solution. The electrical potential of the ISE depends on the activity of target ion in the solution, and is measured relative to the reference electrode (which provides a constant and sample-independent electrical potential). Many efforts have been directed towards development of point-of-care potentiometric ISEs. In addition to the ISE, miniaturization of the reference electrode is also required to accomplish a device that is truly suitable for point-of-care applications. To develop a miniaturized solid-contact reference electrode suitable for point-of-care ion sensing, lipophilic electrolytes was suggested to be used in the reference membrane. The lipophilic electrolyte slowly and continuously leaches out to the solution, and defines the phase boundary potential. The amount of leaching is small enough that it does not significantly change the activity of the lipophilic electrolyte in the membrane, thus, a constant potential is maintained. Due to the lipophilic nature of these electrolytes, they can interfere with the response of ionophore-doped membrane-based ISEs. This work quantifies the amount of leaching of the lipophilic electrolyte to the sample solution over time, and investigates how this leaching interferes with the response of the ISEs.

Keywords: Ion Selective Electrodes
Application Code: Bioanalytical
Methodology Code: Electrochemistry
FT-IR, FT-Near IR and FT-Raman are commercially successful mature technologies dating back to about 1970. However, new developments in Digital Signal Processing, low cost high speed computing, optical coatings and components, detector arrays and software are opening opportunities to apply this technology to new applications. We have generated a temporal and quantitative prediction of the current and future of these technologies and their applications.
Single Bounce ATR accessories for FTIR instruments have become one of the most popular mid-infrared techniques for characterizing materials. High quality spectra can be obtained from milligrams of sample in less than a minute with minimal sample preparation or cleaning. While many of the international Pharmacopoeia organizations have provided clear guidance in qualifying FT-IR instruments for transmission analysis, there is presently very little discussion on how to qualify the instrument with an ATR accessory. In fact the general section of the most recent US Pharmacopeia describes the problems with qualifying an ATR measurement. There is also some debate whether an instrument with a dedicated ATR accessory falls under Instrument Qualification or System Suitability since the choice of ATR internal reflectance element (IRE), angle of incidence and number of reflections can significantly change the resulting infrared spectrum. In this presentation we will discuss some of the causes of variance and why the criteria set for transmittance spectroscopy are not necessarily appropriate for ATR spectroscopy. We will suggest some metrics that might be included in a SOP for qualifying a specific ATR configuration on an FT-IR instrument.

**Keywords:** FTIR, Infrared and Raman, Pharmaceutical

**Application Code:** Pharmaceutical

**Methodology Code:** Molecular Spectroscopy
Fourier Transform Spectroscopy is Alive and Well

The death of FTS was predicted as far back as the early seventies. This notion was related to the loss of some of the advantages commonly cited for FTS to such techniques as laser spectroscopy. The most important advantages frequently cited are the multiplex advantage and the throughput advantage. But using state of the art highly sensitive detectors may limit the signal to noise ratio to the shot noise of the input radiance. It increases as the square root of the total input radiance flux and negates the multiplex advantage. The throughput of an FTS system may not be limited by the interferometer but rather by the detector size or sample size or other geometric optical limitation. Hence it may also be negated. The laboratory FTS (FTIR) is successful and thriving because it provides high sensitivity and accuracy with low cost and easy to use room temperature detectors in combination with high throughput and multiplexing. In remote sensing applications there is more emphasis on the use of sensitive detectors because of the low input radiance flux. Spectrometers with array detectors can be as sensitive as or more sensitive than FTS depending on spectral region and other considerations. An advantage that has gained strength with technical improvements is the precision advantage. In remote sensing it is frequently necessary to compare a spectrum with a line by line calculation based on laboratory measured data bases. This presentation will discuss the merits of remote sensing FTS including satellite based systems.

Keywords: FTIR, Molecular Spectroscopy
Application Code: Environmental
Methodology Code: Molecular Spectroscopy
### Abstract

**Title:** Infrared Spectrometers and Analyzers

**Author:** Arno Simon, Bruker Optik GmbH

**Co-Author(s):** Roland Harig

**Abstract Text:**

Infrared Spectroscopy is increasingly used as sensing technology for monitoring and control in and outside the laboratory. FT-IR is one major technology for recording information in a wide spectral range. The once huge and delicate spectrometers are now shoe-box sized and can be operated as a sensor either in a stand-alone configuration or integrated as OEM sensor in automation or sensing equipment. Examples for use of infrared spectrometers in air monitoring/surveillance, healthcare/life science and other areas will be shown.

**Keywords:** FTIR, Infrared and Raman

**Application Code:** General Interest

**Methodology Code:** Molecular Spectroscopy
Proteins are complex macromolecules that require a comprehensive analysis. The current technology does not fully address the analysis of critical quality attributes (CQA’s) for proteins. We have developed a Best-in-Class High Throughput-Developability and Comparability Assessment (HT-DCA) platform to evaluate proteins in solution. This platform technology takes advantage of: [1] Quantum Cascade Laser (QCL) microscope with enhanced SNR, [2] innovative accessories and cells that allow for the study of an array of proteins under thermal control and [3] automated IR spectral analysis providing a highly informative and statistically robust solution. Hyperspectral Images (HSI) within the MID IR spectral region 1750 - 1000 cm⁻¹ were acquired. The spectral data were then subjected to 2D IR correlation spectroscopy to define the molecular mechanism of aggregation or thermal unfolding of the protein of interest. Consequently, allowing for the determination of aggregation prone regions and domain stability of the protein. Furthermore, Co-distribution correlation analysis was also applied to provide the representative regions of the protein that were affected by the thermal stress based on the distribution population of proteins that were analyzed. Three case studies that involved different proteins and peptides will be presented as proof-of-concept. Among them: [1] NIST mAb standard, [2] protein-peptide interactions and [3] monoclonal antibody (mAb) fragments.

Significance. Our innovative HT-DCA platform is based on first principal and label free, independent of the proteins molecular weight or modification. This platform technology requires minimal protein sample, while providing fast data acquisition times, & ease-of-use. We envision the use of this platform technology for pre-clinical therapeutic protein selection designed for the treatment of diseases, thus ensuring the quality of life of patients.

Keywords: Biopharmaceutical, Microscopy, Protein, Vibrational Spectroscopy
Application Code: Bioanalytical
Methodology Code: Vibrational Spectroscopy
Novel analytical tools based on nanoscience and technology are emerging as methods of choice for analyzing cellular materials, spanning nucleic acids, proteins and enzymes, and small molecules. In particular, the spherical nucleic acid, or SNA, platform, which is characterized by efficient, single-entity cellular uptake, enhanced target hybridization, resistance to enzymatic breakdown, and low immune response, has led to novel extra- and intracellular schemes that have ushered in new paradigms in biodiagnostics, rivaling PCR and ELISA. Further, SNAs are the basis for NanoFlares with have switched the focus from the analysis of external cellular markers to internal genetic ones, especially in single live cells, including circulating tumor cells and stem cells. Another formulation of the NanoFlare platform – the Sticky-flare – can be used to tag RNA sequences for real-time tracking and localization. Likewise, nanopatterned surfaces, especially those composed of extracellular matrix (ECM) proteins and peptides, can be employed to measure and manipulate cellular content. When polymer pen lithography (PPL) is used to precisely tailor the surface chemistry of a substrate, these surfaces can be utilized to immobilize cells and direct cell culture fate. The coupling of this system with self-assembled monolayer matrix-assisted laser desorption ionization (SAMDI) mass spectrometry (MS) allows molecular screening and discovery to be achieved quickly and easily with high sensitivity and validity. As a whole, such nanotechnology-enabled systems are offering unprecedented information about the cellular environment – both healthy and diseased cells – and are leading to important insights in cellular biology and oncology as well as important applications in drug and pharmaceutical screening, clinical science, life science research, and beyond.

Keywords: Biomedical, Biosensors, Nucleic Acids, Wet Chemical Methods
Application Code: Biomedical
Methodology Code: Chemical Methods
This talk will describe an approach for using mass spectrometry to analyze molecular arrays. The arrays are prepared by immobilizing small molecules, proteins, peptides and carbohydrates to self-assembled monolayers of alkanethiolates on gold. This arrays are then treated with reactants—either chemical reagents or enzymes—and then analyzed using the SAMDI technique to identify the masses of substituted alkanethiolates in the monolayer and therefore a broad range of reactivities and post-translational modifications—including kinase, protease, methyltransferase and carbohydrate-directed modifications—and for discovering chemical reactions. This talk will describe applications to high throughput experiments, including the discovery of reactions, the use of carbohydrate arrays to discover novel enzymes, the preparation of peptide arrays to profile the enzyme activities in cell lysates and high-throughput screening to discover novel reactions and small molecular modulators. These examples illustrate the broad capability of the SAMDI method to profile and discover molecular activities in the molecular sciences.

Keywords: Mass Spectrometry, Material Science, Protein

Application Code: Bioanalytical

Methodology Code: New Method
The analysis of heterogeneous ensembles of rare cells requires single-cell resolution to allow phenotypic and genotypic information to be collected accurately. We developed a new approach, magnetic ranking cytometry, that uses the magnetic loading of individual cells to be monitored as a means to report on levels of proteins and nucleic acids at the single cell level. This approach can be used to profile circulating tumor cells in blood and provides a high-information content liquid biopsy in a single measurement.
Metals are essential for all forms of life, and the traditional view of this bioinorganic chemistry is that mobile fluxes of redox-innocent metals like sodium, potassium, calcium, and zinc are used as dynamic signals while redox-active transition metals like copper and iron must be buried and protected as static metabolic cofactors to prevent oxidative stress. We have identified a new paradigm of transition metal signaling, using copper and iron as primary examples to show how such elements can influence neural circuitry and regulate fundamental behaviors such as eating and sleeping. This presentation will focus on our latest efforts to study metals in neurobiology using activity-based sensing (ABS) approaches for imaging and proteomics of dynamic transition metal pools.
Pulsed voltammetry has been used to differentiate serotonin from catecholamines dating back to the early 1980s and including work by Ralph Adams. While techniques like differential (normal) pulse voltammetry provide excellent chemical selectivity, each scan takes tens of seconds (or more) to complete. Thus, these methods are not well suited for detecting rapid neurochemical signaling events in vivo that occur on second to ms timescales. We have adapted a rapid pulse voltammetry and partial least squares regression (PLSR) analysis approach used in multi-electrode systems (electronic tongues), which are employed to detect unknown compounds in complex environments, such as waste water or spoiled food. Using a short series of highly selective pulses (2 ms/pulse, 6-8 pulses, 10 Hz), we can differentiate serotonin and dopamine over a wide range of physiologically relevant concentrations. Pulses were selected to exploit small differences in analyte oxidation and reduction profiles. Data across each pulse is used in PLSR obviating the need to wait for capacitive current to decay after each voltage change, and eliminating background subtraction and problems associated with drift. To realize this strategy, we designed and built instrumentation, and wrote custom analysis software. We are using this approach to distinguish other combinations of neurotransmitters (in the presence of interfering substances), and are deploying this strategy to investigate serotonin and norepinephrine, which are difficult to detect and to differentiate in vivo.


Keywords: Bioanalytical, Microelectrode, Neurochemistry, Voltammetry
Application Code: Neurochemistry
Methodology Code: Electrochemistry
A new approach in public health epidemiology utilizing chemical profiling of wastewater for health biomarkers has been recently pioneered to provide near real-time measurements of public health. Although still in its infancy, this approach, also known as wastewater-based epidemiology (WBE), is currently used to determine community-wide illicit drug use trends via the analysis of urinary drug biomarkers in wastewater with the usage of cutting-edge chemical and bio-analytical techniques. Research has been undertaken by the SEWPROF European Consortium (www.sewprof-itn.eu) and COST Consortium (www.score-cost.eu) to identify and validate new specific biomarkers in order to provide comprehensive information on community-wide health and improved risk assessment with the ultimate goal of disease prevention. This talk will introduce the concept and its rapid advances. It will explore new avenues in the utilization of WBE in the assessment of population health and health risk prediction including (i) a new analytical framework for verification of biomarkers of exposure to chemicals combining human biomonitoring and wastewater fingerprinting with high resolution mass spectrometry – case study of endocrine disruptors in household chemicals, (ii) chiral fingerprinting for verification of chemical potency and route of disposal, (iii) water profiling for biomarkers of community-wide disease status and (iv) linking community-wide pharmaceutical use with environmental health - antibiotics and AMR case study.

**Keywords:** Chiral Separations, Environmental Analysis, Liquid Chromatography/Mass Spectroscopy, Supercritical Application Code: Environmental Methodology Code: Liquid Chromatography/Mass Spectrometry
Assessing Community-Wide Health Via Sewage Wastewater

Wastewater-Based Epidemiology for the Rest of Us: GC-Based Methods for the Routine Monitoring of Illicit Drugs in Wastewater

Despite the multiple advantages of liquid chromatography-tandem mass spectrometry in the analysis of drug biomarkers in aqueous samples, gas chromatography-mass spectrometry (GC/MS) remains the workhorse of many environmental and public health monitoring laboratories. This presentation will describe efforts to develop and validate GC-based methods (MS and MS/MS) for the routine determination of an array of illicit drug biomarkers in municipal wastewater. Biomarkers considered include those for cocaine, amphetamine, methamphetamine, ecstasy, marijuana, fentanyl, and heroin. The pros and cons of different derivatization approaches (including acylation, alkylation, and silylation) will be discussed, as will comparisons against current LC-based methods in terms of cost, analysis time, robustness, and "green" factor.

Keywords: Drugs, Environmental/Waste/Sludge, Gas Chromatography/Mass Spectrometry, GC-MS
Application Code: Environmental
Methodology Code: Gas Chromatography/Mass Spectrometry
Urban Metabolism Metrology (UMM) utilizes the chemical and biological information inherent to wastewater to inform on consumption patterns of participating communities. This may include diet and nutrition, illicit/prescription drug use, alcohol and nicotine consumption, exposure to toxic chemicals and infectious diseases, and population responses to stress. Data obtained from UMM provide community and government entities with near real-time metrics to evaluate changes in population trends, thereby hastening the response to public health emergencies, and supplementing traditional public health programs. This study sought to employ UMM in the United States in participating communities during 2016. Twenty-four hour composite samples were collected monthly from participating wastewater treatment facilities, and analyzed via liquid chromatography tandem mass spectrometry for a suite of 25 chemicals. Through this analysis, cotinine, a metabolite of nicotine consumption, showed promise as a potential population size estimator. Data also revealed annual and seasonal variation of exposure to certain antimicrobials and preservatives, including triclosan, triclocarban, and a number of parabens. Intercommunity comparisons demonstrated a significant difference in per-capita alcohol consumption (p<0.001). While some target drugs such as fentanyl and heroin either degrade or are detected intermittently in trace concentrations, their metabolites were observed to be more stable, providing better estimates of parent drug quantities consumed rather than disposed of. Current work focuses on a comparison of UMM results to orthogonal information sources on public health, including data from law enforcement and healthcare.
Assessing Community-Wide Health Via Sewage Wastewater

Establishing What Else the Analysis of Wastewater Can Reveal About Communities?

Wastewater is known to contain the accumulated biomarkers of endogenous human metabolism that directly reflects the exposure and stressors placed upon all those in a contributing community. Quantitatively measuring these specific biomarkers in wastewater collated from defined communities allows the averaged patterns of exposure and/or effect to be evaluated with the potential to provide excellent spatial and temporal coverage. Thus far the quantitative analysis of wastewater for specific human biomarkers (so-called wastewater-based epidemiology (WBE)) has primarily focused determining the level of community drug use but there is also the clear potential to develop a range of innovative techniques as a solution to quantitatively assess patterns of other factors within populations, such as chemical exposure, nutrition and disease. Our research has to date demonstrated that WBE can already provide ecological data on the prevalence of community licit and illicit drug use, exposure to pesticides and the combined community oxidative stress response. Application of the margin of exposure (MOE) approach has allowed the assessment of the risks posed to specific populations from a number of these exposures, for example alcohol. Importantly we are now also able to accurately estimate catchment populations that clearly facilitates the potential of WBE to provide data on the community level exposure to a much wider range of chemicals (e.g. flame retardants and personal care products), to provide information on nutritional status, a range of health effects (e.g. asthma, allergies, depression), diseases (e.g. cancer, diabetes, obesity) as well as a whole range of infectious disease agents (pathogens such as bacteria, viruses and protozoa) including antibiotic resistance. Early data indicate that WBE used in conjunction with a broad network of wastewater sampling stations and sample archiving can allow for the improved assessment of community health.

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Keywords: Environmental, Environmental Analysis, Organic Mass Spectrometry, Water
Application Code: Environmental
Methodology Code: Mass Spectrometry
Assessing Community-Wide Health Via Sewage Wastewater: An Immunoassay for Measuring 8-Isoprostane in Sewage Wastewater to Gauge Community-wide Health

The chemical composition of a sewage wastewater stream can provide insight to the collective health of communities served by a treatment facility. Monitoring sewage for chemical information has been termed “sewage chemical-information mining” (SCIM). BioSCIM is the application of SCIM for the measurement of biomarkers. BioSCIM can be thought of as a community urinalysis. The occurrence of compounds such as biomarkers of stress or disease can be used to study collective human health. Analytical methods for this application should be relatively inexpensive and provide a high sample load with minimal sample preparation. Immunoassays have demonstrated these attributes for various compounds. Free radicals are generated by normal metabolism, and by exposure to exogenous stressors such as environmental toxins. Oxidative stress occurs when there is an overabundance of damaging free radicals within the body and is an indicator of health. Isoprostanes are key urinary biomarkers to measure oxidative stress in clinical research. The measurement of isoprostanes in sewage wastewater may provide an overall assessment of the level of oxidative stress for a community. To evaluate this idea, samples were collected from a municipal wastewater treatment facility at various stages of the treatment process. Immunoassay methods for determining isoprostanes in urine were obtained for developing application to wastewater samples. Samples were filtered and further processed using a C18 solid-phase extraction and clean-up. Some of the samples caused clogging of the gravity-fed column and required a mild suction for complete elution. Interestingly, this was not dependent on where the samples were obtained along the multi-step wastewater treatment process. Low ng/mL concentrations could be determined in the overall wastewater matrix. A second immunoassay is being explored to further reduce sample processing to rapidly provide data for human exposure assessment studies.

Keywords: Bioanalytical, Environmental/Biological Samples
Application Code: Environmental
Methodology Code: Chemical Methods
Bioanalytical Microsampling with Direct MS Determinations

Microsampling and Cartridge Technologies for Biomedical Mass Spectrometry Analysis

Mass spectrometry is known for its wide applicability with excellent sensitivity and selectivity. Application of mass spectrometry for clinical (especially point-of-care) analysis is a major trend in the development of this technology and will bring a suite of new tools for the biomedical studies. Mass spectrometry could be subjected to severe matrix effect problem and therefore careful sample preparation and chromatographic separation have been the essential steps in the analytical procedures. The transfer of mass spectrometry to POC analysis, however, calls for a major simplification of the procedure as well as a direct interpretation of the results. The combination of the ambient ionization and miniature mass spectrometer has been a promising direction in the development. Direct sampling ionization is enabled with the ambient ionization, bypassing the sophisticated sample preparation traditional used in lab. Critical instrumental development has been made to allow the transfer of ions produced in ambient environment into the trap mass analyzer in vacuum of miniature mass spectrometer for analysis, which enabled the coupling of ambient ionization source with the instrument. For analysis of biofluid or tissue samples, the sample amounts could be significantly reduced, the sampling procedures could be minimally invasive and disposable cartridges could be designed to replace the in-lab equipment used for sampling and cleanup. The high quantitative performance, however, could only be obtained with accurate control of the sample amount and/or the mixing ratio for internal standards and target biomarkers. A set of technologies explored for the microsampling for quantitation using MS will be reported.

Keywords: Biomedical, Instrumentation, Mass Spectrometry
Application Code: Biomedical
Methodology Code: Mass Spectrometry
Bioanalytical Microsampling with Direct MS Determinations

Rapid Metabolomic and Lipidomic Analyses Utilizing Ion Mobility Spectrometry

Due to the numerous extractions required to capture the various small molecules of interest in complex biofluid and environmental samples, there is an enormous need for rapid analytical sampling methods. Small molecule analyses also present numerous analytical challenges such as isomeric indistinguishability and inadequate throughput of measurements. Ion mobility separations (IMS) minimize these limitations by providing high throughput structurally informative analyses, and when combined with mass spectrometry (MS) measurements, the multidimensional IMS-MS analyses provide in depth characterization of each small molecule. However, ionization suppression is readily observed in IMS-MS direct injection studies of complex samples due to the numerous components and high salt concentrations in both biofluids and environmental samples. Thus, a rapid separation is often needed prior to the IMS-MS analyses for high molecular coverage and sample cleanup. This presentation will report on the analysis of biofluids and environmental samples utilizing an automated solid phase extraction (SPE) prior to IMS-MS measurements in order to reduce ionization suppression, quickly remove salts, and detect endogenous and exogenous metabolites from picomolar to millimolar concentration levels.

Abstract Text

Keywords: Automation, Bioanalytical, Mass Spectrometry

Application Code: Biomedical

Methodology Code: Mass Spectrometry
Bioanalytical Microsampling with Direct MS Determinations

Towards a Rapid Diagnostics Tool via Solid Phase Microextraction-Mass Spectrometry (SPME-MS)

The development of new analytical technologies capable of providing high quantitation performance while delivering simplified and fast analysis of biological samples can undoubtedly impact the precision and efficiency of biological investigations in drug-development and point-of-care (POC) diagnosis. In this study, we present diverse SPME-based devices recently developed in our laboratory for the extraction/enrichment of analytes of interest from small volumes of complex sample matrices, which can be directly coupled with mass spectrometry instruments for rapid analysis. These technologies include Coated Blade Spray (CBS), SPME-transmission mode-direct-analysis-in-real-time (SPME-TM-DART-MS) and SPME- microfluidic open interface (SPME-MOI). Total analysis time did not exceed 5 minutes and sample volumes ranging between 1-100 µL were used. Sampling/sample-preparation is performed either by spotting the sample onto the SPME-device, or by immersing the SPME-device on a vessel containing the sample. Despite short extraction times, limits of detection in the sub-ng/mL range were obtained, while good accuracy, and linearity were attained for all the studied probes (e.g. therapeutic-drugs, drugs of abuse, and immunosuppressants) in the diverse matrices scrutinized (e.g. urine, plasma, blood and saliva). In addition, this work puts particular emphasis on the development of novel strategy that allows reaching lower limits of quantitation without affecting the total analysis time or compromising the extraction efficiency by SPME. Finally, this work also describes exemplary cases in which the mere coupling of SPME to MS is not sufficient to answer relevant analytical questions and the use of strategies that allow for removal of co-extracted interferences or source artifacts, such Ion Mobility and Multiple Reaction Monitoring with Multistage Fragmentation (MRM3), are also discussed.

Keywords: Clinical/Toxicology, Mass Spectrometry, Quantitative, SPME
Application Code: Bioanalytical
Methodology Code: Mass Spectrometry
Bioanalytical Microsampling with Direct MS Determinations

Novel Sample Preparation Methods for Metabolic Screening Using Direct Infusion Mass Spectrometry: Electroextraction and Gas-Pressure Assisted MicroLLE

There is an increasing demand for miniaturized, automated, robust sample pretreatment procedures that can be easily connected to direct-infusion mass spectrometry (DI-MS) in order to allow the high-throughput screening of drugs and/or their metabolites or endogenous metabolites in complex samples like plasma or cell extracts.

Liquid–Liquid extraction (LLE) is a common sample pretreatment technique often used for complex aqueous samples in bioanalysis. As there were no automated and miniaturized LLE procedures suitable for small-volume samples using direct infusion mass spectrometry was not available, we have developed a fully automated micro-LLE technique based on gas-pressure assisted mixing followed by passive phase separation, coupled online to nanoelectrospray-DI-MS. This technique works well for a wide range of compounds with acceptable analytical performance.

Often metabolites are (or can be) charged. We have developed for these analytes an additional strategy, three-phase electroextraction: analytes are extracted from a liquid aqueous sample donor phase through an immiscible organic solvent layer acting as a filter phase into a liquid aqueous acceptor phase by the application of an electric field between the donor and acceptor phase. The organic filter phase acts as a purification filter, which prevents, for example, proteins from migrating into the acceptor phase. The acceptor phase can be a droplet hanging from a (conductive) pipet tip in the organic phase. After enrichment of metabolites, this droplet was subsequently infused into the mass spectrometer. We have applied this technique to human plasma for various metabolite classes.

An outlook is given on high throughput sample preparation for analysis of small molecules in various type of samples.

Keywords:
- Mass Spectrometry
- Sample Handling/Automation
- Metabolomics
- Metabonomics

Application Code:
- Genomics, Proteomics and Other 'Omics

Methodology Code:
- Mass Spectrometry
Novel monolith materials were prepared for solid phase microextraction (SPME) of some herbicides in water samples, then the SPME was directly coupled to plasma assisted laser desorption / ionization mass spectrometry (PALDI-MS) without chromatography separation. The detection sensitivity was below 0.01 ng/mL, and the analytical time was within 3 minutes.

**Keywords:** Detection, Environmental/Biological Samples, Mass Spectrometry, Solid Phase Extraction

**Application Code:** Bioanalytical

**Methodology Code:** Sampling and Sample Preparation
Adulteration of foods for economic gain has been a problem for centuries. A wide variety of foods have been targeted. Honey and maple syrup are well known examples of high value commodities that are attractive for adulteration. However, other foods, such as lemon juice, grated cheese, protein powder, and others have also been targets for adulteration. Adulteration of any food not only defrauds the consumer, but also hurts legitimate producers of these products through unfair competition and falsely low prices. In addition, the adulterants used may pose unseen risks to the consumer and may thus endanger the public health.

Over the years, we have received a wide variety of samples in our laboratory for determination of adulteration. Some examples of these include determination of polysaccharide contamination in protein powder, added starch in grated cheese, added citric acid in lemon juice, and dilution of maple syrup and honey with sugar syrups. For these determinations, we have utilized a multi-faceted approach to uncover the presence of a possible hidden adulterant. Methods include ion chromatography, and isotope ratio mass spectrometry, among other methods. We will explore some examples of analyses, discuss the challenges and celebrate the successes.

Keywords: Food Science, Forensic Chemistry, Ion Chromatography, Isotope Ratio MS
Application Code: Food Science
Methodology Code: Liquid Chromatography
Honey is a multibillion dollar per year high-value commodity traded on a global scale. In 2016, the US produced over 160 million lbs and imported about 370 million lbs of honey to meet demand. Honey adulteration is an ancient problem with new wrinkles. Advances in sugar syrup production from corn starch in the 70’s yielded syrups with sugar profiles very similar to those in honey. The perfect honey adulterant! Fortunately, the fractionation of the stable isotopes of carbon by physical and biochemical processes provides a tool for detection of certain forms of adulteration.

Flowering angiosperms which provide nectar for honeybees are exclusively "C3" plants with carbon isotope ratios around -25 to -30 which results in similar values for honey produced from those nectars. The global average for honey is about -25.5 with a standard deviation of about 1 permille. Commercially important "C4" plants (eg., corn, cane, sorghum) have isotope values around -12.

The gross adulteration of honey by a syrup derived from C4 plants is easy enough to detect simply as a weighted mixing model between C4 and C3 end members. However, the natural variability of honey results in a broad range for the honey end member values – and the calculation of relative contribution is only as accurate as the end member values.

Fortunately, honey contains a natural protein fraction consisting mainly of the enzymes responsible for the honey “ripening” process. The protein fraction acts as an internal standard, representing the value close to that of the unadulterated honey in question for a more accurate calculation of the addition of C4 sugars to a honey. The AOAC 998.12 method involves the comparison of the ratio of the bulk honey to the ratio of the extracted protein to calculate the apparent C4 sugar contribution.

Keywords: Food Contaminants, Food Identification, Isotope Ratio MS
Application Code: Food Science
Methodology Code: High-Energy Spectroscopy
Defending Against Food Fraud: Utilizing Old and New Technologies in the Fight Against Adulteration

Utilizing Emerging Technology and Methods to find EMA in our Food System

We never imagined we’d have to worry about someone tampering with our food supply. Today we are faced with new stories each day of intentional contamination of our food for economic gain. To have fraudsters target our food supply...the consequences are devastating. We cannot opt out of eating which makes our food supply a critical infrastructure. A significant amount of research and development has been done to improve our ability to combat food system disruptions. This session will highlight capabilities and methods developed to identify drivers/pre-cursors of Economically Motivated Adulteration as well as emerging events.

Primary Author: Amy Kircher
University of Minnesota

Co-Author(s)

Abstract Text

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Session Title
Defending Against Food Fraud: Utilizing Old and New Technologies in the Fight Against Adulteration

Abstract Title
Utilizing Emerging Technology and Methods to find EMA in our Food System

Abstract Text

Keywords: Consumer Products, Contamination, Data Analysis, Data Mining
Application Code: Food Safety
Methodology Code: New Method
Edible oils refined from special commodities such as rice bran and camellia (tea seed) have higher economical values and are susceptible to adulteration. Traditional identification of edible oils using fatty acid composition is not sufficient to defend food adulteration. To ensure the safety, quality and integrity of edible oils, we identified marker compounds or compound ratio specific in some of the edible oils. The marker compounds/compound ratio, along with other identification methods, will add extra protection against food adulteration.

Keywords: Food Contaminants, Food Identification, Food Safety, Validation
Application Code: Food Safety
Methodology Code: Gas Chromatography
Defending Against Food Fraud: Utilizing Old and New Technologies in the Fight Against Adulteration

State-of-the-Art Techniques for the Authenticity Testing of Honey, Agave Syrup and Beeswax

The analysis of food authenticity is the constant competition between counterfeiters and authenticity analysts. On the one hand, people with economic motivation try to market a food more favorably. On the other hand, researchers and lab service providers are constantly looking for new analytical approaches in the form of targeted counterfeiting indicators or the fingerprint. Thus in principle, authenticity testing techniques are based on targeted or non-targeted methods, either by means of single parameter methods or analysis combinations. Solutions and implementation are illustrated by the examples honey, agave and beeswax.

In the field of carbohydrate-based foods, e.g. honey, agave and other syrups the falsification is usually performed with cheaper carbohydrates. These sugar syrups are made by C4 or C3 plant species such as beet, wheat, rice or corn and are much cheaper than honey and therefore preferably used to increase desired profit margins. Highly sophisticated analytical methods are absolutely necessary to detect exogenous sugar, protein or hydrocarbon additions. According to the current state-of-the-art, targeted methods and non-targeted screening methods (e.g. NMR, EA/LC-IRMS, FT-IR, LC-ELSD) are used to detect all known and yet unknown adulterants with acceptable sensitivity and accuracy.

In principle, non-targeted screening methods are based on an authentic reference database to generate a unique fingerprint, targeted methods are based on specific marker compounds for the added syrup. In this presentation, pros and cons of different authenticity testing methods are presented and - based on a few examples - a comparison is given between non-targeted and targeted analysis results. In summary, the targeted- and non-targeted techniques both contribute to a significant improvement of the authenticity testing of honey, agave and bee wax. They are important for the elimination of adulterated products from the market or already before entry into the market.

Keywords: Food Identification, FTIR, Isotope Ratio MS, NMR
Application Code: Food Identification
Methodology Code: Liquid Chromatography/Mass Spectrometry
Therapeutic proteins are miracle drugs for millions of patients globally. Unfortunately, however, with many of these products a relatively large fraction of patients experience loss of efficacy, due to adverse immunogenicity. Studies for more than 50 years in humans and animal models have documented that aggregates and particles are important causes of immunogenicity. Every step in the life history of a protein product can cause aggregation and particle formation, from initial fermentation to final delivery to patients. Fully understanding the impacts of these steps on product quality requires sensitive and robust methods for characterizing and quantifying nano- and microparticles. Such data are particularly important for bioprocessing steps. As an example, this presentation will show results for commercial filling pump operation using a peristaltic pump and three different brands of commercially used tubing. One highlight from the study was that pumping a protein solution through Pharmed tubing resulted in much lower microparticles levels than pumping through Accusil or Masterflex tubing. But nanoparticle levels in samples pumped through Pharmed tubing were much higher than those observed in samples pumped through the other tubing brands. Furthermore, with an accelerated degradation method of post-pumping agitation, it was seen that the high level of nanoparticles resulted in very high concentrations of microparticles. Effects of formulation pH and surfactants were also tested and results indicated that both nano- and microparticle measurements are crucial for understanding fully the impact of these solution conditions. Finally, light obscuration was also used to measure microparticles and it was found that this method was not suitable to detect and quantify increases in protein particles resulting from filling pump operation or post-pumping agitation.
Large molecule biotherapeutics are inherently complex with myriad modifications that affect their safety and efficacy. They are manufactured in living mammalian cells in bioreactor and developing an optimal cell culture bioprocess for the production of biopharmaceuticals requires routine monitoring of medium conditions such as carbon source (glucose), nitrogen source (glutamine) and various other biologically important compounds such as vitamins, nucleic acids and other primary metabolites. To meet the demand for comprehensive multi-component analysis of medium component, we have optimized the analytical conditions and developed a high-throughput LC/MS/MS method that can monitor relative abundance of 95 compounds. Using this multi-attribute method, we demonstrated the change in abundance of culture medium components associated with hybridoma growth over a period of 5 days.

Antibody based biotherapeutics also require their PK/PD to be determined to establish their safety and toxicity profiles. Antibody bioanalysis is complicated, tedious with many timed steps that include antibody capture from complex plasma matrix, digestion, cleanup prior to analysis. To simplify this workflow, Shimadzu recently developed an innovative nanotechnology based surface and molecular-orientation limited proteolysis or nSMOL Antibody Bioanalysis platform for the quantitative analysis of antibody drugs in biological fluids by LC/MS/MS. This sample preparation platform is a multi-product platform that perform selective proteolysis of only the Fab region of monoclonal antibodies therefore increasing the detection sensitivity of surrogate peptides in CDR regions that can be accurately quantified via MRM measurements using a triple quadrupole high performance liquid chromatograph mass spectrometer.

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

**Application Code:** Bioanalytical

**Methodology Code:** Liquid Chromatography/Mass Spectrometry
Drop Coat Deposition Confocal Raman (DCDCR) Spectroscopy: This Sleeping Beauty is Now Awake for Protein Characterization With a “Coffee Ring”

Drop Coat Deposition (DCD) is a pre-concentration sampling technique that is known to increase the signal-to-noise (S/N) ratio in Raman spectroscopy. In spite of improved S/N ratio, this technique has been underutilized in the past for protein structural characterization. This pre-concentration technique has been extensively used in this study to produce protein deposits in the “coffee ring”. The protein in these deposits were found to be well hydrated, thus maintaining the protein in its native structural form. The confocal Raman microscope provided highly resolved visual image of the “coffee ring” and thus offered the spatial resolution to collect the Raman spectrum of the protein from the ring in its native state. High quality Raman spectra from model proteins and biologics drug substances and drug products were collected and the Amide I vibrational band, which is typically in the region of 1620 to 1720 cm⁻¹ was used to determine the secondary structure distribution of proteins. This technique has also been evaluated to monitor protein degradation, such as acid, heat, and disulfide reduction. This talk will feature all the above applications of DCD technique coupled with confocal Raman spectroscopy.

Keywords: Bioanalytical, Biopharmaceutical, Biospectroscopy, Raman Spectroscopy

Application Code: Pharmaceutical

Methodology Code: Molecular Spectroscopy
The maintenance of an appropriate intracellular environment is a constant challenge for all living organisms, from prokaryotes to multicellular eukaryotes. Intracellular homeostasis is maintained by membrane proteins, e.g., membrane transporter, transporting various compounds such as ions, sugars, amino acids, and drugs across the biomembrane. Therefore, the analysis of transmembrane transport is crucial to understanding cell physiology as well as for exploring the bioavailability of drugs. Although extensive studies have performed to elucidate the mechanism of membrane transport, quantitatively and reproducibly measuring the transport in a high throughput format has remained difficult due to the complexity of processes involved in membrane formation. Here, we address this issue by developing a novel artificial biomembrane microsystem (ALBiC) that forms sub-million femtoliter reaction chambers, each sealed with an artificial biomembrane with an efficiency of over 90%. Due to the infinitesimal volume of these chambers, ALBiC can enhance the detection sensitivity by a factor of $10^6$, demonstrating the single-molecule analysis of membrane transport in a high throughput manner. Moreover, we have recently demonstrated some physiological membrane aspects on ALBiC, such as asymmetric transbilayer phospholipid distribution, and modulation of membrane potential across lipid bilayers. Thus, our new platform, ALBiC, holds promise for understanding the mechanism of membrane transport under semi-physiologic conditions as well as for further analytical and pharmacological applications.

Keywords: Biopharmaceutical, Lab-on-a-Chip/Microfluidics, Membrane
Application Code: Bioanalytical
Methodology Code: Microfluidics/Lab-on-a-Chip
The volume magnetic susceptibility of particles had the highly possibility of practical particle analysis. The particle volume magnetic susceptibility was calculated by the magnetophoretic method which was observed individual particle migration velocity around high magnetic field gradient area[1~3]. Heretofore, as a living cell evaluation method, a method of measuring the amount of synthesizing DNA using a marker as an indicator of cell proliferation and a method of obtaining the reduction amount of coenzyme (NADH), that is, the amount of ATP by using a reductive color developing agent were used. In this study, we report the evaluation of yeast cells using their volume magnetic susceptibility as an examination method of novel cell activity. On the other hand, we also developed a simple particle size measuring device "Nanomeasure". This is a device for trapping particles made of two glass plates, and it is possible to align particles for each particle size by trapping the particles in a small gap between the glass plates. Nanomeasure can measure not only general industrial products such as toner and ink, but also living cells and exosome.

References

Keywords: Analysis, Bioanalytical
Application Code: Bioanalytical
Methodology Code: Process Analytical Techniques
Self-assembling protein microarrays arrays can be used to study protein-protein interactions, protein-drug interactions, search for enzyme substrates, and as tools to search for disease biomarkers. In particular, recent experiments have focused on using these protein microarrays to search for autoantibody responses in cancer patients. These experiments show promise in finding antibody responses that appear in only cancer patients. New methods using click chemistry-based reagents also allow the application of these arrays for discovering new substrates of post translational modification.
Identifying changes in animal models of disease at the earliest time point prior to pathology is desired because these alterations are more likely to cause the pathology and be successful drug targets. At these early time points, however, alterations in protein expression may be small and difficult to identify hidden by the overwhelming static proteome. One possible solution is to identify only newly synthesized proteins (NSP) within a discrete time period. Quantifying the NSP proteome is analogous to quantifying translation rates. The analysis of NSP, however, has largely been restricted to cultured cells. We developed PALM (Pulse Azidohomoalanine Labeling in Mammals) analysis to identify and quantify NSP from rodent tissues by mass spectrometry.

Azidohomoalanine (AHA) is modified methionine that is accepted by the endogenous methionine tRNA and insert into proteins in vivo. AHA can be covalently linked to a biotin alkyne through click chemistry. Thus, AHA proteins or peptides can be enriched and efficiently separated from the whole proteome through avidin bead enrichment. A special rodent diet was developed where methionine was replaced with AHA. To quantify the NSP, a heavy biotin-alkyne was synthesized. To test this novel quantitation tag, differences in NSP in liver tissue induced by the transgenic removal of liver kinase B1 (LKB1) were quantified. To demonstrate usefulness of the heavy biotin alkyne tags, one KO and one WT liver were analyzed three times independently to test for technical reproducibility. The heavy/light AHA peptide ratios were highly correlated ($r > 0.8$) between these replicates. When peptide ratios were averaged for NSP ratios, the correlation coefficient between the p A heavy stable isotope labeled version of AHA has been synthesized to provide the ability to quantitate newly synthesized proteins and this will be discussed.

Keywords: Bioinformatics, Liquid Chromatography, Liquid Chromatography/Mass Spectroscopy, Proteomics
Application Code: Genomics, Proteomics and Other 'Omnics
Methodology Code: Liquid Chromatography/Mass Spectrometry
A premise of precision health is that continuous individual health monitoring will allow early disease detection, early intervention, increased quality of life and reduced health costs. Cardiovascular disease (CVD) remains a leading cause of mortality worldwide. Atherosclerosis, a primary CVD risk factor, begins early prior to clinical signs. Early and selective intervention is effective at reducing CVD burden but early signs of major adverse cardiac events (MACE) are often missed. To address this need, we are i) developing diagnostic markers for early atherosclerosis and ii) implementing remote monitoring and sampling platforms for the continuous surveillance of mid-risk individuals to predict MACE, including heart attack or stroke. First, we have analyzed 100s of aortic and left anterior descending coronary arteries obtained from individuals (<50 years old) using DIA and DDA mass spectrometry (MS) discovery workflows. Using convex analysis of mixtures and differential dependent network modeling we defined the composition, network re-wiring and regulatory features of early atherosclerosis. Early atherosclerosis tissue-secreted proteins were quantified using targeted protein MS multiplex assays in plasma of individuals with varying degrees of CVD. Second, using volumetric absorptive microsampling devices that allows for remote blood collection and our automated MS sample preparation and assay workflows we are carrying out continuous patent-centric biomarker assessment of 240 mid-risk CVD patients. We are comparing continuous physiological biometrics, patent reported outcomes, classical (BNP/cTnI) and novel protein biomarkers (10 or 400 proteins) with an in-depth 1153 lipid panel to understand the impact of disease on biological variability and that will predict MACE.

**Keywords:** Bioinformatics, Biomedical, Biosensors, Mass Spectrometry

**Application Code:** Biomedical

**Methodology Code:** Mass Spectrometry
Abstract Text

The most popular bottom-up proteomics workflow uses trypsin to enzymatically cleave proteins C-terminal to lysine and arginine residues prior to LCMS/MS analysis of the resulting peptides. The high frequency of lysine residues in particular can sometimes lead to the formation of peptides too small or uninformative for optimal analysis. Moreover, analysis of very complex peptide mixtures means that many peptides are not effectively sampled, leading to patchy sequence coverage and incomplete characterization of mutations and post-translational modifications. We have developed ultraviolet photodissociation as a new activation method for fragmentation of biomolecules, and this MS/MS method have proven useful for analysis of peptides and proteins. We have implemented UVPD into bottom-up, middle-down, and top-down workflows to extend the versatility of mass spectrometry-based proteomics, and we have integrated UVPD with auxiliary methods such as selective derivatization and proton-transfer reactions to further expand the performance metrics of UVPD in the arena of high throughput proteomics applications.

Keywords: Mass Spectrometry, Protein, Proteomics

Application Code: Bioanalytical

Methodology Code: Mass Spectrometry
Here, we demonstrate a new type of proteome analysis that utilizes an all-native approach to preserve several kinds of protein information completely lost in denaturing proteomics when performed as per the current state-of-the-art. Applying this new method in discovery mode to human cell lines and mouse heart tissue, we are able to characterize previously unreported protein complexes, metal-ion cofactors, and redox-sensitive modifications as well as validate the high fidelity of the approach with dozens of known cases. The new information garnered by Native Proteomics reports far more directly on the actual forms of endogenous proteins and protein complexes present within cells and tissues than denaturing methods (Figure 1, right).

In contrast with denaturing proteomics, the central value proposition of Native Proteomics is that it preserves those labile structures and assemblies that are formed and present in vivo, but lost upon denaturation. Using Native Proteomics in untargeted mode, we report 125 multi-proteoform complexes and 217 proteoforms, a major change for the previously targeted world of native mass spectrometry. We posit that the value of these observations transcends traditional proteomics and reshapes our fundamental view of mammalian proteome complexity. Current methods for interrogation of complex proteomes disrupt these interactions, due to denaturation and/or digestion, necessitating an extra step of inference that blurs or completely erases the information now shown to be within our grasp. Importantly, the readout of modification occupancy and complex subunit stoichiometry with high fidelity greatly clarifies which sources of protein-level variation are in fact present and at what abundance. It follows that such a novel readout of metal cofactors, enzyme-bound intermediates, glycosylations, and prevalent modifications of exposed cysteines will have a major impact in determining functional forms that regulate key processes in cells and tissues.

Keywords: Proteomics
Application Code: Genomics, Proteomics and Other 'Omics
Methodology Code: Mass Spectrometry
Neurotransmission at Single and Nano-Resolved Bio-Structures  

Electroanalysis at the Single Cell and Subcellular Level

Several types of amperometric nanosensors have been developed for electrochemical experiments at single mammalian cells, bacteria and biological vesicles. These nanoelectrodes can be used as the scanning electrochemical microscopy (SECM) tips to penetrate biomembranes and carry out spatially resolved experiments in biological systems. Such experiments can enable the detection and quantitation of miniscule quantities of analytes produced in living cells. The focus of this paper is on microscopic understanding of the nanosensor responses with the ultimate goal to improve the sensitivity and selectivity of single cell electroanalysis. The applications range from dopamine analysis to intracellular measurements of the reactive oxygen and nitrogen species to detection and identification of single bacteria by electrochemical collision technique.

Keywords: Bioanalytical, Electrochemistry, Nanotechnology, Voltammetry

Application Code: Bioanalytical

Methodology Code: Electrochemistry
Carbon nanomaterial electrodes have been developed because they improve the sensitivity, selectivity, and electron transfer kinetics of electrodes. Our lab has recently explored carbon nanotubes, carbon nanospikes, and carbon nanohorns as new electrode materials for neurotransmitter detection. In general, these nanomaterials add surface area and surface roughness without greatly enlarging the overall size of the electrode. Electrode treatments can also be used to enhance the properties of these electrodes, particularly those of carbon nanotube fibers and yarns. The other area of exploration is the use of carbon nanopipette electrodes, which offer a small size and high sensitivity. The nanopipette electrodes can be used to make spatially resolved measurements of neurotransmitters in small organisms, such as the fruit fly.

Keywords: Electrochemistry, Neurochemistry, Voltammetry
Application Code: Bioanalytical
Methodology Code: Electrochemistry
Nanoscale in-vivo studies on the release of neurotransmitters are required to understand brain function and disease. Acetylcholine, the first neurotransmitter identified in 1914, plays key role in human health and its defects have been associated with neurodegeneration. Elucidating its release dynamics and concentration profiles at the source of its release, the synaptic cleft, is instrumental in understanding neurodegenerative diseases. Here we present our study of acetylcholine synaptic release with a nanoelectrode of ~15 nm in radius, unveiling unprecedented details on its release dynamics and concentration profiles around single synaptic cleft. We employed nanoresolved scanning electrochemical microscope (SECM) (1, 2, 3, 4) to position the nanoelectrode around the synaptic cleft.


Keywords: Bioanalytical, Electrochemistry, Electrodes, Neurochemistry
Application Code: Neurochemistry
Methodology Code: Electrochemistry
In this presentation, we discuss about an application of nanoscale scanning electrochemical microscopy (SECM) to image single biological nanopores. Specifically, we will image single nuclear pore complexes that perforate the nuclear envelope, which is isolated from a biological cell and spread over a solid substrate. Individual nanopores with inner and outer diameters of ~50 nm and ~120 nm, respectively, are resolved. SECM Imaging of the individual nanopores in a physiological buffer reveals the permeability of a nanopore is related to its topography. A "plugged" nanopore is actually as permeable as an "open" nanopore, thereby indicating that the plug is a part of the transport or a highly permeable substance that is trapped in the pore.

**Keywords:** Electrochemistry, Microelectrode, Microscopy, Nanotechnology

**Application Code:** Bioanalytical

**Methodology Code:** Electrochemistry
In this talk we will establish that one may extract extremely important information about fusion pore sizes and dynamics as well as about the internal structure of the so-called dense core matrix of endocrine cells. In particular, we will establish that the classical textbook (and unfortunately research) ‘Full Fusion’ paradigm is not relevant to most of release events by endocrine cells. At this stage we believe that full fusion events monitored by TIRFM do not correspond to the release of neurotransmitters as postulated but rather feature a follow-up stage that may happen if the vesicle does not close, through which the chromogranins may be released to be digested into peptide hormones. We will also report on the monitoring of intra-synaptic vesicular release and show that in this case the fusion pores behave in a completely different manner than for endocrine cells.

Keywords: Electrochemistry, Microelectrode, Neurochemistry
Application Code: Neurochemistry
Methodology Code: Electrochemistry
The identification of fragmentary and/or commingled human remains following a mass disaster or discovery of a mixed grave is an immediate obligation of the forensic community, not only to decedents’ families, but also to aid investigators and document evidence for the criminal justice system. Such incidents as airline crashes, infrastructure failures, industry explosions and natural catastrophes can result in large numbers of fragmentary human remains spread over large areas of land or water. These remains may be burned, contaminated with caustic or other substances (e.g. jet fuel and industrial chemicals), or subject to natural taphonomic processes that destroy tissue or inhibit the DNA polymerases that are used in DNA identity testing.

Proteins, which are more abundant and less sensitive to degradation than DNA, offer an alternative bio-identity molecule when DNA is degraded or polymerases inhibited. Recent advances in mass spectrometry instrumentation make it faster, simpler, more accurate and more sensitive than earlier instruments. Similar advances have occurred in the power of bioinformatics to rapidly search and recover annotated protein information from ever increasing databases. These advances have propelled proteomics to the forefront of basic research and pharmaceutical discovery. These same mass spectrometry tools are directly applicable to the forensic identification of individuals.

Here we demonstrate the use of proteomic mass spectrometry and bioinformatics for human identification. Using tissue samples from 14 individuals from the University of Tennessee Forensic Anthropology Research Facility (IRB approved) we have identified 13 muscle protein polymorphisms, from among hundreds of proteins, that are present at frequencies greater >5% and can be used for individual identification. All polymorphisms were confirmed by DNA sequencing. Advantages and challenges of human identification through proteomic mass spectrometry will be discussed.
We have been investigating the potential of epigenetic methylation as a procedure for the identification of body fluids and age at crime scenes. Epigenetic methylation generally appears in the form of methylated cytosines in the genome. These modified bases are typically present in the form of clusters of CpG motifs upstream from gene transcription sites where they are used to control gene expression.

Diagnostic epigenetic loci are typically located using whole genome array studies which are then followed by quantitative pyrosequencing. Because methylation information is lost following the PCR, extracted samples are subjected to bisulfite modification which converts unmethylated cytosines into thymines following PCR. Pyrosequencing is next used to provide a quantitative estimate of the level or percent methylation at each CpG site. In addition we have explored the use of real time PCR with high resolution melting capability for bulk analysis of these sites.

There are a number of advantages of using epigenetic loci in body fluid identification. These include the fact that samples can be extracted using standard procedures. Because the methylation involves covalent bonding, the method is very stable, permitting analysis of samples over 20 years of age. The procedure is also human specific and resistant to inhibition. Furthermore, epigenetic loci may also be used reveal phenotypic information such as age and behavior. This occurs due to the fact that certain CPG loci can be influenced by random or environmental processes, resulting in correlations with age or other behavior such as smoking. In this presentation we will discuss our development of epigenetic loci for body fluid and age determination. This will include our work in the analysis of methyl array data, pyrosequencing, real time PCR, and the initial validation of these procedures.

**Abstract Text**

**Keywords:** Bioinformatics, Biological Samples, Biotechnology, Forensics

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

**Methodology Code:** Capillary Electrophoresis
Short tandem repeats (STRs) have a higher mutation rate than single nucleotide polymorphisms (SNPs). STR genotyping is highly informative for genetic applications such as DNA fingerprinting. However, there is little informative genetic analysis of the haplotype structure of STRs and SNPs. This knowledge gap mainly due to the absence of high-throughput technology for these loci.

Despite their wide application, the analysis of STRs with next generation sequencing (NGS) methods is limited by several major issues including: i) Only the reads encompassing an entire STR locus are informative; ii) PCR amplification during library preparation can introduce artifactual “stutter” mutations that confound accurate genotyping. Consequently, analysis on STRs requires more sequencing depth with finely controlled target selection, but current methods such as bait-hybridization are of limited utility. Here, we developed a novel targeted sequencing technology (STR-Seq), which can simultaneously genotype thousands of STR loci and phase proximal SNPs with significantly higher accuracy than all other methods. STR-Seq uses single-molecule sequencing in combination with targeted in vitro CRISPR-Cas9 fragmentation.

In this study, we evaluated 2,543 STR loci from a population of 1,004 individuals. Our analysis included 436 Marshfield loci, which has been used to characterize the population sample, and an additional 1,915 loci where a proximal SNP was positioned within 100bp of the STR. Overall, we identified approximately 2,000 STR genotypes and 1,000 STR-SNP haplotypes per individual. STR-SNP linkage was extremely low (mean $r^2 < 0.1$). We discovered a new class of STRs which are highly polymorphic as noted by having 20 or more alleles. Finally, we identified a significant number of STR-SNP haplotypes and describe the geographical and population differences of these previously undescribed, novel genetic markers.

**Keywords:** Bioinformatics, Biotechnology, Forensics

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

**Methodology Code:** New Method
DNA re-identification is used for a broad suite of applications, ranging from cell line and tissue authentication to crime scene sample identification. However, current re-identification schemes suffer from high latency. We developed a rapid, and portable strategy to re-identify human DNA, called “MinION sketching”. Using data from Oxford Nanopore Technologies’ sequencer, MinION sketching requires only 3min of sequencing and 60-300 random SNPs to identify a sample, enabling near real-time applications of DNA re-identification. Hands-on preparation of the samples can be reduced to <1 hour. We re-identify individuals using sparse reference files as generated by Direct-to-Consumer companies. Our method potentiates application of MinION sketching for border control, on-site crime scene re-identification of DNA samples and rapid identification of victims after a mass disaster.

Keywords: Bioinformatics, Biological Samples, Forensics
Application Code: Homeland Security/Forensics
Methodology Code: Portable Instruments
The evaluation and interpretation of forensic DNA mixture evidence faces greater interpretational challenges due to increasingly complex mixture evidence. Even with an increased number of CODIS core markers, mixture interpretation is challenging. Massively parallel sequencing (MPS) is a technology that can facilitate mixture interpretation and simplify some complex mixtures. The value of MPS is its ability to provide sequence data in addition to the nominal length of STR alleles. This project focuses on defining current STR marker variation, identifying novel STRs that may be better suited for mixture deconvolution, and development of software tools to facilitate allele calling of STRs typed by MPS. The repeat and flanking regions of 27 autosomal, 7 X-chromosome and 24 Y-chromosome STR markers were characterized in 777 unrelated individuals from four population groups. Additionally novel STRs are being mined from public databases that may facilitate mixture deconvolution. To be able to identify sequence variation within an amplicon software needed to be developed. STRait Razor is a bioinformatics suite used to identify and characterize sequence and length-based polymorphisms in MPS data. STRait Razor consists of two major components: The STRait Razor perl script and the Strait Razor Excel workbook collate, annotate and visualize haplotypes. The latest version provides both a stable code-base that operates on all major operating systems including Microsoft Windows and an indexing strategy tailored to the identification of sequence variants based on anchor sequences. The newest version is 660 times faster than previous versions and maintains all the features as the previous versions.

Keywords: Bioinformatics, Biotechnology, Genomics
Application Code: Other
Methodology Code: New Method
In this presentation, we report some of the latest advances in femtosecond and nanosecond laser 2D and 3D elemental imaging with LIBS. We study the fundamentals of material sampling, including plasma diffusion and mixing across boundaries of inhomogeneous media. We also introduce direct high-aspect ratio laser sampling coupled with wavelength-resolved plasma imaging as a way to expedite the chemical mapping process with LIBS. Finally, we demonstrate these imaging capabilities in energy storage and in plant systems. The ability of LIBS to directly image elements in 3D allows a sophisticated understanding of the distribution of impurities, which can govern the electrochemical behavior of Li-ion battery components, as well as the transport of toxic elements across plants, all of which are not easily observed by other conventional characterization techniques.

Keywords: Electrochemistry, Environmental/Biological Samples, Laser, Ultra Fast Spectroscopy
Application Code: Material Science
Methodology Code: Atomic Spectroscopy/Elemental Analysis
A fast trending trend in medicine and biomedicine is the use of elemental maps or elemental image with the final purpose of understand elemental behavior and migration in live individuals. Laser-ablation coupled with ICP-MS improved the methodology to recreate isotopic maps from tissues, human or not. Lately the high cost related to this technique have been overcome by commercials LIBS systems, opening the access to similar images with a lower cost. Taking the advantage that the elements of interest are in concentration superior to trace element, this technique provides the sufficient sensitivity to be quantitative. A growing application for this methodology is the study of elemental composition in human tissue. Metallic nanoparticles have an extensive use in medical treatments, health applications as well as hygiene. However, their impact and how is possible to track this material inside human body is a challenge. A sample set of human tissues was exposed to heavy metals and mapping using a commercial LIBS system to know the distribution of this material, and understand its migration and interaction in the body. As another field of application, anthropology benefits from LIBS when it provides major and minor elements distribution present in organs such as human teeth. Quantitative concentration maps are possible using element-doped hydroxyapatite pellets as standards to quantify all the elements of interest. This can be used as a way to know more about diet, social status and habits for cultural anthropological studies.

Keywords: Atomic Emission Spectroscopy, Forensics, Medical
Application Code: Other
Methodology Code: Atomic Spectroscopy/Elemental Analysis
The imaging capability of laser-induced breakdown spectroscopy (LIBS) has a high potential in various domains including biology, industry, geology and medicine (figure 1). This approach can be distinguished by its ease in use, multi-elemental capability, detection of light elements, as well as operation at ambient conditions. This is furthermore the only all-optical technique providing space-resolved elemental information with ppm-scale sensitivity and μm-range resolution. Compared to other elemental imaging methods, such as laser ablation inductively coupled plasma mass spectrometry or synchrotron X-ray fluorescence, LIBS has the advantage of a fast operating speed (100 Hz and potentially higher) allowing to provide large-scale microscopic images in reasonable time periods. These advantages, in particular the all-optical design and the fast operation speed, make LIBS very attractive to be used in research laboratories for routine investigations.

However, advanced technological solutions must be found for this application since elemental imaging requires high sensitivity, sharp spatial resolution, high speed of acquisition as well as the ability to process a huge quantity of data. In this presentation, we will summarize the progresses made in our teams in the last few years concerning the implementation of the LIBS imaging. After describing the measurement principle, we will describe the main “keys” for the instrumentation as well as the data analysis. Different examples will be then shown with the idea to illustrate the specificities of LIBS among other elemental imaging approaches, such as the possibility to detect and image light elements, the coupling with optical and Raman imaging and the analysis of large-scale samples. Different perspectives will be finally proposed.

Keywords: Atomic Emission Spectroscopy, Imaging, Laser, Spectroscopy
Application Code: Other
Methodology Code: Atomic Spectroscopy/Elemental Analysis
Elemental Mapping for Nanoparticle Diffusion in Plant Materials

Laser-induced breakdown spectroscopy (LIBS) has been used directly on agricultural samples [1, 2, 3] for both micro-analysis and elemental mapping [4] to study the distribution of intrinsic elements as well as the diffusion of eventual nanoparticles[5]. LIBS can provide concentration ranges of Zn above 100 ppm. Samples from the plant, are analyzed and mapped quantitatively for Zn (using Zn or Zn+ signals as proxy for ZnO NPs) after in-house calibration. Elemental maps taken at different times after injection provide information about the rate and the processes for diffusion of the NPs.


Keywords: Atomic Emission Spectroscopy, Bioanalytical, Food Safety, Plasma Emission (ICP/MIP/DCP/etc.)
Application Code: Bioanalytical
Methodology Code: Atomic Spectroscopy/Elemental Analysis
Detailed geological models and mineralogy knowledge greatly assist the mining industry in locating mineral resources, and can reduce the need for expensive drilling, saving time and money. LIBS imaging provides a highly selective and sensitive means of gaining this information from materials and drill cores derived from drilling. Until recently, geologists, and geotechnical engineers had to manually interpret and record minerals found in these materials. This manual method – the visual interpretation of drill cores – is labor intensive and subject to human error. LIBS technology enables a systematic and selective way – based on the physics of atomic emission spectroscopy (AES) – to minimize or eliminate human error via automation. When bench-marking this drill core analyzer against existing technology, it is possible to see the significant advantage that can be achieved with this technology in the mining industry. First, LIBS imaging provides the highest selectivity for elemental imaging through atomic emission spectroscopy. Second, the surgical resolution of laser ablation, the ease of scanning a large area and adapting to any sample shape and size, makes the instrument the best solution for permanent core logging. Hence, logged data can be used to discover new exploitable mines in the future using data mining, when other element demand becomes economically viable to extract. LIBS logging technology has the potential to eliminate the need for keeping drill core samples in core shacks as prescribed by the authorities. The results obtained using this drill core analyzer enable the logging of 200 to 1000 meters of drill core samples per day. The results presented in this paper demonstrate that minuscule gold nuggets can be easily out-lined on drill core samples coming from different types of mineralogical assemblages. The high throughput analysis demonstrated here showcases LIBS logging technology as a potential game changer in the field of mining exploration.

Keywords: Atomic Emission Spectroscopy, Elemental Analysis, Imaging, Instrumentation
Application Code: Other
Methodology Code: Atomic Spectroscopy/Elemental Analysis
Abstract Text
Nanocrystals have been proposed as useful alternatives to traditional dyes used in cell imaging due to their resistance to photobleaching, strong response to two photon excitation, and the ability to conjugate a variety of targeting molecules. Silicon (Si) nanocrystals in particular have fluorescence lifetimes on the order of 10's of microseconds making them candidates for imaging techniques that make use of the fluorescence lifetime. We have investigated the feasibility of using Si nanocrystals and fluorescent dyes that emit in the same spectral region (e.g. 600-700 nm) with fluorescence lifetime imaging microscopy (FLIM), using a straightforward gating separation of the short lifetime signal (~ 2-5 ns, fluorescent dye) from the long lifetime signal (10's of µs, silicon nanocrystals). Using a single two-photon excitation source at 800 nm, the fluorescence from two dyes (one blue emitting and one red emitting) and Si nanocrystals can be measured simultaneously. Spectral as well as temporal multiplexing is used to visualize the localization of the imaging contrast agents within the cells. We have used this technique with various organelle dyes to observe the colocalization of Si nanocrystals in the endosomes of mouse macrophage cells.

Keywords: Biomedical, Imaging, Nanotechnology, Semiconductor
Application Code: Biomedical
Methodology Code: Fluorescence/Luminescence
Silicon nanocrystals (SiNCs) have been attracting attention as active materials in a variety of prototype devices including, solar cells, light-emitting diodes, photodetectors as well as biological and medical imaging. These, and other applications require well-defined materials with predictable properties. Traditionally, SiNC surfaces are rendered processable and stable toward oxidation by employing a variation of the general hydrosilylation reaction; they all involve the addition of a silicon-hydride bond on the SiNC surface across a carbon-carbon double (or triple) bond and affords a “monolayer” attached through a robust silicon-carbon linkage. These derivatization protocols are often very time-consuming, can provide surfaces that are partial oxidized, and even lead to complex oligomeric surfaces. In this context, we have chosen to explore alternative functionalization protocols by employing non-standard reagents that could rapidly provide well-defined surfaces with minimal surface oxidation. This presentation will include a discussion of our recent exploration into SiNC surface chemistry including two new reactive platforms that have opened the door to functionalized SiNCs exhibiting absolute photoluminescent quantum yields approaching those of status quo CdSe-based quantum dots.

Keywords: Imaging, Luminescence, Semiconductor, Sensors
Application Code: Nanotechnology
Methodology Code: Fluorescence/Luminescence
This talk will provide an overview of our group’s efforts to develop photoluminescent silicon quantum dots for biological imaging applications. Silicon quantum dots have generated interest due to the abundance and low cost of silicon, its lack of elemental toxicity, the possibility of achieving emission spanning visible and near-infrared wavelengths, and the ability to covalently attach organic molecules to the silicon surface. In principle, silicon quantum dots could provide most of the advantages of heavy metal based quantum dots, such as CdSe, without many of the potential drawbacks. More than a decade ago, our group developed a combined bottom-up and top-down approach to synthesis of silicon nanoparticles, followed by strategies for surface functionalization, encapsulation, and bioconjugation. By optimizing surface passivation, we were able to achieve photoluminescence quantum yields in the range of 10 to 50%, which is high enough for optical bioimaging. We demonstrated in vivo tumor targeting and sentinel lymph node mapping, and also combined silicon quantum dots with magnetic nanoparticles, dyes, and plasmonic particles to create multimodal imaging agents. In in vivo biocompatibility tests in mice and small primates, we found that the silicon quantum dots were well-tolerated, but that they did not degrade in vivo, even over a period of months. Although silicon itself will degrade to silicic acid in water or biological fluids, by passivating the surface sufficiently to achieve high photoluminescence quantum yield, we prevented degradation. Key challenges in future efforts to employ silicon quantum dots for bioimaging will include development of effective surface passivating ligands that can allow degradation in vivo and new strategies to overcome the inherently low optical absorbance of silicon at visible and near-IR wavelengths.

**Keywords:** Biomedical, Luminescence, Nanotechnology

**Application Code:** Biomedical

**Methodology Code:** Fluorescence/Luminescence
Fluorescence imaging is a powerful research tool used to identify a wide range of abnormalities in tissues, though it has seen only limited use in the clinic. One reason for this is that fluorescence imaging is limited by the strong absorption and scattering of visible wavelengths of light by mammalian tissues. Another reason is that tissues display a native fluorescence that often overwhelms signals from the probe fluorophore. This presentation discusses two features of luminescent silicon nanoparticles that can be used to improve the fidelity and resolution of in vivo imaging: two-photon excitation and time-gated imaging of the long-lived excited state of quantum-confined silicon. The dyes commonly used as NIR or two-photon imaging agents often have limited stability and they can display various toxic side effects. By contrast, a relatively low toxicity silicon nanoparticle shows substantially greater photo-stability under one- or two-photon imaging conditions. Furthermore, the long-lived (microseconds) excited state of silicon enables elimination of interfering background fluorescence by means of time-gated imaging. This presentation will discuss the use of photoluminescent porous Si nanoparticles as imaging agents, and it will also describe how various molecular targeting groups can improve in vivo image fidelity.

Keywords: Biomedical, Biotechnology, Fluorescence, Imaging
Application Code: Biomedical
Methodology Code: Fluorescence/Luminescence
Silicon Nanoparticles for Sensing and Imaging

New Emissive Nanostructures Templated by Silicon Nanotubes

Silicon Nanotubes (SiNTs) are a form of nanostructured silicon with properties of potential merit as an analytical probe, including tunable inner and outer diameter, nanotube length, diverse surface functionalization opportunities, along with novel porous sidewall morphologies capable of nanoscale infiltration and release. Any practical applications require the development of routes to loading their tubular interior as well as functionalization of the outer surface. In this presentation, we demonstrate examples of each: (1) the hollow nanotube interior serves as a nanoscale reaction vessel that can be exploited for the formation of new strongly emissive species whose dimensions are dictated by the nanotube template; (2) sensing and delivery functionalization is made possible by the attachment of selected fluorescent probe molecules to the silicon nanotube surface, as well as the coupling of primary amine moieties for electrostatic nucleotide binding relevant to gene delivery.

For the first category, we describe the controlled formation of organo-lead & tin halide perovskite nanostructures within the hollow SiNT interior, whose emission wavelength is not only dictated by perovskite composition but also strongly influenced by the structure of the nanotube. Encapsulation by the nanotube strongly impacts the phase purity and long-term stability of the perovskite structure as well. In the second category, nanotube surface functionalization opens new opportunities for concomitant fluorescent imaging and/or attachment of probes for cellular recognition specificity. In addition to structural characterization by electron microscopies (SEM, TEM) and X-ray diffraction, the fundamental luminescence/fluorescence of these modified Si NTs, including confocal fluorescent imaging, is described.

Keywords: Gene Therapy, Luminescence, Nanotechnology, Semiconductor
Application Code: Nanotechnology
Methodology Code: Fluorescence/Luminescence
The exploitation of natural sources and later synthetic molecules to generate blue to purple coloration in textiles has a long history in the dyer’s craft. Natural indigoids such as indigo, woad, and Tyrian or shellfish purple served this purpose for millennia, but in the late 1800s synthetic analogs, in particular indigotin, quickly replaced natural indigoids. Halogenated versions of the dye were also created, and some like 5,5′-dibromoindigo were apparently brought to market, but have not been significantly discussed in the literature or found in forensic and technical art history investigations of textiles. This paper reports the first identification in a museum context of this unusual synthetic brominated analog of indigo, discovered on two mid-20th century Japanese yukata (Figure 1.) Full analytical data collected on reference materials using liquid chromatography-mass spectrometry, UV-visible spectroscopy, Raman microspectroscopy, Fourier transform infrared spectroscopy, and X-ray fluorescence spectroscopy are provided to assist with future identifications of this relatively unknown colorant. Density functional theory applied to 5,5′-dibromoindigo was used to confirm the experimental Raman spectra and to refute the possibility that the yukata dyes could be the now unavailable 4,4′- or 7,7′-dibromoindigo analogs.

Abstract Text

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Keywords: Art/Archaeology, Chromatography, Identification, Liquid Chromatography
Application Code: Art/Archaeology
Methodology Code: Molecular Spectroscopy
Textiles, being organic materials, have survived since antiquity in only a few places on earth, namely those which lack the necessities, such as water or oxygen, of degradative microorganisms. Most other textiles have decomposed and disappeared. Thus, our knowledge of the dyes used to color ancient textiles is limited to specimens from relatively few locales, such as very arid places, peat bogs and a few others. We have focused our work on yellow dyes because there are more yellow colorants than for any other color (there is a single blue, maybe a dozen reds and hundreds of yellows). Since yellow colorants are plentiful, the plants that produced them tend to be locally available, so identification of the dye (usually a mixture of yellow compounds) often gives clues as to where the textile was dyed. During the past 15 years or so, we have analyzed over 100 yellow dye-producing plants using HPLC with diode array and mass detection, enabling us to characterize each component by HPLC retention time, electronic spectrum and molecular mass (and sometimes fragments). We have used this information to identify the plants, or plant types, used to dye ancient textiles (i.e., >1000 years old) or others for which no historical record exists. As a result, some patterns are beginning to emerge. Protoberberine and flavone C-glycoside dyes were often used in the Far East, pagoda tree (Sophora japonica) buds in China, yellow larkspur (Delphinium semibarbatum) in Central Asia, plants containing chalcones and flavonol sulfates in the Andean regions of South America and flavones (e.g., from Reseda luteola) in Europe and the Middle East. Although flavones, which are relatively stable to, were often used, we have found that many cultures have used flavonol 3-O-glycosides, which are also light-stable, for yellows. Characterization of flavonoid glycosides has been possible only since the advent of mass spectrometry for dye analysis.

Keywords: Liquid Chromatography, Mass Spectrometry
Application Code: Art/Archaeology
Methodology Code: Liquid Chromatography/Mass Spectrometry
Dyers and craftsmen have harnessed the coloring strength of insects and plants to dye textiles since the dawn of mankind. Hence, naturally the first applications of surface-enhanced Raman spectroscopy (SERS) to the analysis of organic colorants in the cultural heritage field have been directed to dyed textiles. As early as 1987, Guineau and Guichard identified the red anthraquinone dye madder on a woolen thread using a roughened silver electrode as a SERS-active probe. Since then, the field of SERS for the analysis of textile dyes has grown enormously. Continued research over the course of the past decade has led to the construction of comprehensive spectral databases of dyes; to the evaluation of ad-hoc sample treatment methods and analytical protocols; and to the comparative study of the efficiency and performance of various metal substrates. In addition, recent literature has described instrumentation and technique advancements aimed at solving the unique challenges posed by the analysis of irreplaceable objects, namely, quasi non-destructive sampling, spatial resolution improvement, and resolution of dye mixtures. Highlighting the main advantages and limitations of SERS when applied to the analysis of textile dyes, this talk will review the most salient methodological and technological milestones that have traced the history of the technique in curatorial and conservation-based research to date. Case studies presented will include an extensive work for the scientific analysis of the organic colorants used in a selection of Kaitag textiles, a unique embroidered textile art form from the Kaitag district of Southwest Daghestan, Russia; and a survey of natural and synthetic dyes found in 19th-century Navajo blankets from the Art Institute of Chicago that, along with a detailed technical examination of the yarns’ physical attributes, allowed researchers to reassess the interpretation and dating of some of the weavings.
In 1856, the first synthetic dye was obtained via serendipity by William Henry Perkin leading to the development of synthetic colorants, which have become the dominating dyestuffs used in the industry. Yet in 2018, despite the impact of 150 years of synthetic dye research on society and the environment, no freely available comprehensive digital or physical database of dyes exists. A cheminformatics dye resource would enable advancement of basic and applied research and development. In 2014, Eastman Chemical Company donated a collection of ~98,000 synthetic dyes to the College of Textiles at North Carolina State University. This donation provides the perfect opportunity to create the valuable and much-needed database that dye researchers need. We have digitalized a subset of 3200 dyes which allowed us to start the constitutional and structural analysis of the collection using cheminformatics approaches. In addition, a systematic study via tandem mass spectrometry of different dyestuffs allows a better understanding of the multiple fragmentation pathways of dyes with different chromophores, isomers, and substituents (e.g. electron donating or electron withdrawing groups). Tandem mass spectrometry experiments will also allow us to create a mass spectra database that can be used for identification of unknown colorants or give us a better idea of the chromophores present in a dye molecule of a museum artifact.

Keywords: Chemometrics, Database, Mass Spectrometry, Tandem Mass Spec
Application Code: Other
Methodology Code: Mass Spectrometry
For more than a decade at the Canadian Conservation Institute (CCI), natural dyes from historical objects have been analyzed using gas chromatography-mass spectrometry (GC-MS). A key first step in the procedure is extraction using the alkaline derivatizing agent [(trifluoromethyl)-phenyltrimethylammonium hydroxide (TMTFTH). Subsequent analysis by GC-MS allows not only for the identification of colorants, but also dye degradation products, non-dye marker compounds, dye bath auxiliaries, and substances present on the object through original use or later contamination. An important complement to the method is the use of ion extraction and deconvolution software (such as AMDIS) with purpose-built mass spectral search libraries. This tool enables the operator to extract small target peaks at specific retention indices, which greatly increases the sensitivity of the method and rapidly highlights peaks of interest. The presentation will provide an overview of the methodology and discuss the compounds identified through the reactions of TMTFTH with reference materials and samples from historical objects. The natural dyes that will be discussed include: flavonoid dyes (e.g., old fustic, dyer’s buckthorn and red sandalwood), quinone dyes (e.g., madder, [i]Galium[/i] and butternut), indigoid dyes (e.g., indigo and Tyrian purple) and some lichen species. Furthermore, the presentation will demonstrate how the identification of dyestuff is possible based on non-dye marker compounds that are unique to certain plants. One of the next steps in the on-going research is the adaptation of the methodology for synthetic dyestuffs. Preliminary research into the identification of early synthetic dyes using GC-MS will be introduced.

Keywords: Art/Archaeology, Data Analysis, Gas Chromatography/Mass Spectrometry, Identification
Application Code: Art/Archaeology
Methodology Code: Gas Chromatography/Mass Spectrometry
Innovative Ways to Engage Students in Analytical Chemistry

Just-in-Time Videos and Mini-Case Studies to Engage and Prepare Students for a Classic Quant Lab

Our Quant Lab is classical. Students are given an unknown for which they must determine the concentration of one or more components. All marks are on the accuracy of their analysis. Being off by as little as 2% results in a failed lab. We take this approach to prepare students for the real world, where mistakes can have dire consequences. But while this is an important learning opportunity, students are understandably terrified. Recently we have explored two initiatives to enhance student learning and confidence, and reduce their in-lab stress.

Firstly, videos are an effective way to supplement classroom learning and help students understand concepts, retain information, and draw connections. Fifteen short (<5 min) instructional videos were created on techniques such as weighing, pipetting and reading a buret (https://tinyurl.com/yaq7uwg6). Specific focus was given to instructing proper analytical techniques essential for students’ laboratory success. This presentation will share our observations on students’ use of these videos and how it impacted student confidence and performance in the lab.

Secondly, if a student receives a failing grade in a lab, the student does a post-mortem with the instructor to determine the cause of the failure. Historically about 80% of these post-mortems identify the fatal error. This exercise enables the student to avoid making the same blunder again. But students only learn what they did wrong after they fail. And not all students fail, and so not all get to share in this valuable learning experience. We have developed mini-case studies, which are faux lab notebook pages for a failed experiment. In lecture, groups of students do a post-mortem of the faux lab to discover the fatal error (only to discover that there is usually more than one thing that went wrong). The outcomes of these mini-case study exercises will also be shared in the presentation.
Innovative Ways to Engage Students in Analytical Chemistry

New Uses for Video Conferencing: Bringing the Lab to the Classroom, and the Student to the Study Session

There are many challenges to teaching larger and larger class sizes. Some are immediately obvious, such as diminished direct interaction with individual students, and challenges in addressing a broader range of student needs. Less obvious are the challenges of time and space associate with large class sizes. How do you schedule office hours, or study sessions to accommodate so many different schedules? How can you do demonstrations in class, if the infrastructure even exists, so that they can be seen by all?

At San Diego State University professor Harrison is incorporating video conferencing as a tool to help overcome these barriers in his large classrooms (500 students). With a lecture hall design that lacks all means of supporting chemical demonstrations, video conferencing is used to pull the lab into the classroom. The video conference allows the assistant in the lab to conduct experiments in realtime, while the video is projected in the classroom, and can even be seen on laptops, tablets, or phones. This gives the demonstration added intimacy, as well as added learning opportunities not provided by recoded videos (e.g. YouTube), as the experiments may not go exactly as expected.

Outside of the classroom the video conferencing can be used as a means of expanding the reach of office hours and study sessions. By allowing students to join these sessions by video the barrier of getting to the meeting is removed, allowing students greater ease in accessing the information and resource that they need.

This presentation will outline how professor Harrison has implemented these tools, as well as how they have been used, and perceived by his students.

Keywords: Education, Teaching/Education
Application Code: Other
Methodology Code: Education/Teaching
After teaching Instrumental Analysis laboratory for several years, I reflected on whether students were meeting course learning objectives and developing skills needed for the job market. While most students were fairly competent in preparing solutions and calculations, assignments in the course did not address critical skills related to literature searching, experiment design, troubleshooting, and communicating science to different audiences. Laboratory projects in collaboration with local businesses and schools were developed to address these deficiencies and promote excitement for the topic. An external collaborator introduced each project to the class which changed my role as a professor from evaluator of student work to facilitator and consultant. In a project with Baxter Biopharma Solutions, students investigated the linearity, detection limit, accuracy, and precision of a Coomassie microplate assay to detect protein impurities in the drug Heparin. Students wrote validation reports and standard operating procedures for their work using the industry format and experts provided feedback on their work. For the Upland Brewery, students analyzed important flavor components of beer and made scientific posters to communicate their results with the brewery staff. To enhance an outreach program in the biology department, students assisted in a plant nutrition lesson and then analyzed plants grown in different soils for nutrient composition. They communicated their results with elementary school students in classroom visits. Details of laboratory projects, strategies for implementing projects in large classes, and results of student surveys will be discussed.
In an active-learning environment, a computer simulation of an analytical system is a useful way to allow theoretical concepts to emerge through a process of guided play. This presentation will consider what makes an effective simulation and present examples of simulations developed in the R programming environment that introduce students to topics covered routinely in quantitative analysis and instrumental analysis with a particular focus on electrochemistry and spectroscopy.
Innovative Ways to Engage Students in Analytical Chemistry

Engaging a Large Analytical Chemistry Class in a Current Research Project Exploring Transformations and Impact of Emerging Energy Storage Nanomaterials

As nanoparticles become increasingly incorporated into consumer products and industrial processes, the potential environmental impacts involved in the disposal of these products are only poorly understood. In this talk, I’ll describe an active learning lab project developed for an undergraduate analytical chemistry class, where student groups explore the release of transition metals from Lithium Nickel Manganese Cobalt Oxide (NMC) particles, which are used in Lithium Ion Batteries (LIBs). Students explore how changing a variable within the solution conditions, such as, pH, presence of phosphate ions, small molecules (glucose or lactose), salinity, or temperature, affect the rate of dissolution of both Co$^{2+}$ and Ni$^{2+}$ from NMC particles into solution. The basis of determination is the complexation of Co$^{2+}$ and Ni$^{2+}$ with dithizone in a micellar medium. The determination of Ni$^{2+}$ and Co$^{2+}$ released from LIBs using a spectrophotometric method provides an opportunity for students to become familiar with complexation chemistry, stoichiometry, and the quantitative analysis of a mixture of analytes. Students also gain hands-on experience with adapting methods from scientific literature, absorbance and emission spectroscopy, and exploring quality control techniques, such as, spike recovery and method detection limits.

This project was adapted from current research supported by the Center for Sustainable Nanotechnology.

Keywords: ICP, Nanotechnology, Quantitative, Spectrophotometry
Application Code: Environmental
Methodology Code: Education/Teaching
Critical-thinking, decision-making, and processing skills (CTDMP skills) are essential in STEM fields; however, few courses offer opportunities for students to practice these skills and receive feedback. We have developed a pedagogical method and a prototype online tool, code-named "Alchemy", that addresses this gap. A pilot study was enthusiastically-received by analytical chemistry students and demonstrated strong potential for teaching/learning gains. We are further developing and evaluating the Alchemy concept through (1) enhancement of the online tool for greater versatility and capability in teaching/learning; (2) new pedagogical content development; and (3) new learning analytics capabilities to support data-enabled pedagogies that guide teaching/learning through metrics of class and individual student performance and attitudes. Alchemy will benefit students through improved CTDMP skills, enhanced appreciation for the complexity of real-world problems, improved discipline-specific skills, and transferable problem-solving skills for future employment. This presentation will address the implementation and evaluation of Alchemy to date, particularly in the context of sophomore-level analytical chemistry, and will also discuss new and ongoing developments in the Alchemy project.
In this work, different techniques are presented to establish the role of dynamin, a molecular motor from the GTPase family, in exocytosis of transmitters or membrane receptors. First, single cell amperometry shows that blocking the GTPase activity of dynamin with the selective inhibitor dynasore inhibited exocytosis. The analysis of the data hints that dynamin contributes to secretion by helping the expansion of the fusion pore.

A hybrid microfluidic/ electrochemical system was then used to detect dopamine from a population of PC12 cells cultured onto the surface of filter paper. Dopamine release after stimulation with acetylcholine was observed in this setup, as well as the effects of the drug L-3,4-dihydroxyphenylalanine. More importantly, the inhibitory effect of dynasore was confirmed at the multicellular level, and the data scales well with the single cell results. Overall, this study demonstrates the validity of this system for chemical analyses at cells or artificial cell constructs, such as organs-on-a-chip.

Finally, this approach was applied to glucose uptake in muscle cells. Dynasore was found to inhibit this phenomenon, most likely the result of the inhibited exocytosis of the specific glucose transporter GLUT4. The nematode C. elegans was then used as an in vivo model. Dynamin inhibition was found to block glucose uptake and to alter the behavioral response of the worms to high-glucose levels, thus establishing the in vivo relevance of the study. Importantly, this finding could be relevant to the mechanisms of diabetes, where the translocation of GLUT4 is impaired.

Overall, these results indicate that electrochemistry can tackle multilevel studies and that dynamin plays a crucial role in membrane expression of transporters and in the release of small messenger molecules.
Nitric oxide (NO) is an important free radical synthesised and released by brain cells. It can modulate synaptic transmission and neuronal network activity but also mediate neuronal injury through oxidative stress. It is believed that NO exerts its physiological functions at low concentrations whereas oxidative stress occurs at much higher levels. However, the quantitative threshold at which NO concentrations become toxic is still poorly defined. Here, we detected endogenous brain NO release using 7 µm diameter carbon fiber microelectrodes first coated with a layer of nickel-porphyrin (Ni-P) and a screening layer of trimethoxymethylsilane. The fluorinated xerogel improved the selectivity of the sensor compared to Nafion, a fluoropolymer commonly used to block interfering molecules. Nitrite, 5-HT and AA amperometric detection was significantly reduced by the silane layer and NO detection was stable over 7 days of storage or throughout a 3h in vivo experiment. In vivo, these electrodes could quantify brain NO release evoked by a toxic local microinjection of the glutamatergic agonist N-Methyl-D-aspartate at 1.33 [0.49-4.93] µM. The amperometric signal was almost completely blocked by the NO-synthase inhibitor 7-nitroindazole. Fluorinated xerogel-coated carbon fiber microelectrodes therefore provide excellent stability, sensitivity and selectivity to detect brain NO and quantify its concentrations. Toxic NO actions like those evoked by the neurotoxin NMDA may take place in the low micromolar range.

Supported by CNRS, Inserm, University of Lyon, and grant FGC46-2016 from Fondations Gueules Cassées.
Obesity is a rapidly growing epidemic affecting over a third of the US population. Human and rat studies suggest that brain regions mediating reward and motivation for food may play a role in overeating, particularly in susceptible individuals. Using rats selectively bred to be prone to obesity, we investigated neurochemical alterations in the nucleus accumbens that may contribute to the likelihood of over-consumption of food and obesity. In vivo microdialysis was used in conjunction with benzoyl chloride derivatization and LC/MS/MS to measure neurochemical responses to sucrose in obesity prone and obesity resistant rats. Ingestion of sucrose caused a greater increase in nucleus accumbens concentrations of glucose, glutamate, glutamine, and GABA in obesity resistant rats compared to obesity prone. Rats were fed 13C6-glucose and both 13C6 and 12C glucose were measured to differentiate changes in exogenous vs endogenous glucose levels. Our findings indicate that changes in brain glucose are a result of both preexisting central glucose and that from the exogenous source. Discrepancies in both the rate and magnitude of neurochemical responses to sucrose in rats prone and resistant to obesity indicate underlying differences in neuronal mechanisms that may contribute to obesity.

This work was funded by T32DA007268, R37EB003320, R01DK106188.
Electrochemistry enables the real-time, continuous monitoring of changes in electroactive species in discrete brain nuclei. For example with sub-second temporal resolution, fast scan cyclic voltammetry (FSCV) enables changes in neurochemical concentrations, such as dopamine, to be correlated with behavioral data. Recent advances by our lab and others have enabled detection of new molecular targets with electrochemistry. This talk will review the progress on creating new electrochemical sensors and schemes for monitoring metabolic-related substrates, such as pH, glucose, lactate, oxygen and blood flow. Recent research has validated a microfabricated electrolytic hydrogen clearance sensor for detecting changes in blood flow. By monitoring hydrogen clearance at a microfabricated collector-generator electrode pair, changes in blood flow can be monitored in tissue. This presentation will describe the validation process for these sensors. In other studies by our group, single carbon-fiber electrodes have been modified with a glucose oxidase (GOx) modified hydrogels enabling changes in glucose availability to be recorded within discrete locations in the brain while maintaining the beneficial properties of voltammetry. The GOx sensor has been used to simultaneously monitor dopamine release and changes in glucose availability during electrical stimulation of the mid-brain. The status of these measurements will be reviewed and future directions described.
Implantable probes are valuable scientific tools for basic neuroscience research and clinical applications. Neurotechnologies provide direct readouts of neurological signal and neurochemical processes. These tools are generally most valuable when performance capacities extend over months and years for the study memory, plasticity, and behavior, or monitoring patient condition. This need has generated a variety of device designs from carbon fibers for FSCV and electrophysiology to microdialysis probes. Regardless of the technology used, the breaching of the Blood-Brain Barrier to insert the devices triggers a cascade of biochemical pathways resulting in a complex molecular and cellular response to the implanted probe. Molecular and cellular changes in the microenvironment surrounding the probe can dramatically impact the neuronal network and signal sensitivity of neurochemical sensors. In turn, studies aimed at detecting subtle plastic changes in the neuronal network to learning/training paradigms can be adversely affected. Our work examines the magnitude, variability, and time course of the dynamic molecular and cellular components of this foreign body response to state-of-the-art carbon fiber biosensor, microfabricated arrays, and microdialysis probes using electrochemistry, immunohistochemistry, electrophysiology, and in vivo multiphotom microscopy. The findings show that the foreign body response dramatically impact signal quality across all neurotechnologies to varying degree across all time points.

**Abstract Text**

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**Keywords:** Electrodes, Fluorescence, Neural Network, Neurochemistry

**Application Code:** Neurochemistry

**Methodology Code:** Sensors
Neurotransmitters act as endogenous chemical messengers in the human body. Within the subdivision of the monoamines, dopamine and serotonin are the two neurotransmitters that are of particular interest in the study of human emotions. One of the most acknowledged methods to sample these mono-amines is microdialysis where we collect dopamine and serotonin [in vivo] from rats that can move around freely within their cage. As a result of their inherent main functionality, both of the above mentioned neurotransmitters are directly affected by behavioural changes. Therefore we have adjusted the rats’ experimental confinement to a degree where behavioural, food reward based, experiments are implemented with direct microdialysis sampling. This presentation will cover the approach to developing this new type of rat cage while also addressing the technological difficulties during the process.

Keywords: Capillary LC, Electrochemistry, Liquid Chromatography, Neurochemistry
Application Code: Neurochemistry
Methodology Code: Liquid Chromatography
The design and optimization of current and new neurochemical technologies necessitates thorough in vivo testing. Technologies such as microelectrode arrays and microdialysis probes provide unique and essential sensitivities to important neurophysiological parameters for a wide range of applications. However, their use in the living brain is often accompanied by trauma or ischemic events that may alter the neurophysiology of interest. Optogenetic tools can be easily coupled with optical microscopy to provide unique opportunities to manipulate and/or record brain activity in vivo in concert with neurochemical probes. These approaches are readily available in rodent models and can be used to establish sensitivity constraints or evaluate the impact of these technologies on brain function in acute or chronic settings. Examples of these two approaches, optical neuro-manipulation and optical neuro-detection, are discussed in the context of implanted microelectrodes. Graded modulation of neuronal activity in broad (or selective) neuronal populations is possible using optogenetic actuators like channelrhodopsin. We demonstrate the use of this tool to determine neuronal viability around implanted microelectrodes. Likewise, optogenetic reporters of neuronal activity like GCaMP can be used to monitor network activity before and after implantation for relatively long time scales. We demonstrate the use of this tool to determine the impact of probe implantation on network activity. These findings highlight the role optogenetic tools can have in helping to guide and tailor the design constraints of current and new neurochemical technologies.

Keywords: Biomedical, Biotechnology, Imaging, Microscopy
Application Code: Biomedical
Methodology Code: Microscopy
Malignant gliomas account for approximately 70% of new malignant primary brain tumor diagnoses each year in the United States. The current standard therapy for newly diagnosed malignant gliomas involves surgical resection, radiotherapy, and chemotherapy. Conventional pressure-driven infusion of molecules into the brain has recently shown much promise, however it has drawbacks such as lack of control of injection and damage from neuronal distortion. By using electrokinetic transport as the driving force for local delivery of solutes to the brain, many of these issues can be circumvented.

Our objective was to demonstrate that electrokinetic convection-enhanced delivery is a viable means for delivery of locally high concentrations of solutes to the brain. Directionality and quantification of the infusate in hydrogel brain surrogates and in living rat brains are demonstrated. Volume of distributions using the fluorescent dye Ru(bpy)₃²⁺ in vivo was 46.1 µl (±2.2 SEM). Control of directional transport was also achieved over distances ranging from several hundred micrometers to more than four millimeter in poly(acrylamide-co-acrylic acid) hydrogels. Concentration profiles were modeled using COMSOL, these finding were in agreement with our in vivo data. This type of delivery, electrokinetic, has the potential to improve the control over drug infusions leading to better clinical outcomes.

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**Keywords:** Biomedical, Electrochemistry, Microscopy

**Application Code:** Biomedical

**Methodology Code:** Sensors
Overview of Recent Developments in Ultrafast Chromatography: From Theory to Applications

Ultrafast High-Throughput SFC for Analysis of Pharmaceutical Compounds and Intermediates

In the last few decades, liquid and sub/supercritical fluid chromatography have been established as essential techniques in the pharmaceutical industry. High-efficiency stationary phases have allowed separation scientists to develop rapid HPLC and SFC separation methods using short columns for screening processes. In this study, we develop ultrafast chromatographic methods using SFC for a variety of pharmaceutically-related drug targets and intermediates. This talk will focus on high-throughput screening method development using high-efficiency stationary phases.

Date: Wednesday, February 28, 2018 - Aft
Time: 01:30 PM
Room: 208B

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Keywords: Chromatography, High Throughput Chemical Analysis, Pharmaceutical, Supercritical Fluid Chromatography

Application Code: Pharmaceutical
Methodology Code: Supercritical Fluid Chromatography
The increasing demand for ultrafast high performance chiral separations has fostered the development of highly efficient packing materials for liquid chromatography (LC). In this talk, we will show our recent achievements in enantioselective Ultra High Performance Chromatography (eUHPC) focusing on brush-type Chiral Stationary Phases (CSPs). A large body of chromatographic data has been obtained using the well-known Whelk-O1 selector. This selector was bonded on sub-2μm Fully Porous, high surface area, silica Particles (sub-2μm-FPP). The potential of sub-2μm FPP-Whelk-O1 CSP has been then investigated in both UHPLC and Ultra High Performance Supercritical Fluid Chromatography (UHPSFC) [1]. The benefit of UHPSFC with sub-2μm FPP-Whelk-O1 CSP for ultra-fast separations is evident as, in the regime of very fast separations, it is possible to achieve larger efficiency and resolution. Simultaneous ultra-fast and high efficiency separations on ultra-short columns (1-cm long) allowed analysis times, in some cases, shorter than 1 second. Parallel investigations have been carried out on 2μm Superficially Porous Particles (2μm-SPP). Covalent immobilization of the glycopeptide antibiotic teicoplanin yielded the new Zwitterionic Teicoplanin (2μm SPP-CSP_Tzwitt). Columns packed with this SPP-CSP exhibited excellent kinetic performance (up to 300,000 N/m) and extremely fast mass transfer, allowing to perform baseline resolutions of several racemates that are completed in the seconds time domain.[2]

References

Keywords: Chiral Separations, Liquid Chromatography, Pharmaceutical, Supercritical Fluid Chromatography
Application Code: Other
Methodology Code: Separation Sciences
Overview of Recent Developments in Ultrafast Chromatography: From Theory to Applications

Ultrafast Enantioselective Chromatography As the Second Dimension in 2D-LC Experiments

Chromatographic separation and analysis of complex mixtures of closely related species is one of the most challenging tasks in modern pharmaceutical analysis. In recent years, two-dimensional liquid chromatography (2D-LC) has become a valuable tool for improving peak capacity and selectivity. However, the relatively slow speed of chiral separations has limited the use of chiral stationary phases (CSPs) as the second dimension in 2D-LC, especially in the comprehensive mode. Realizing that the recent revolution in the field of ultrafast enantioselective chromatography could now provide significantly faster separations, we herein report an investigation into the use of ultrafast chiral chromatography as a second dimension for 2D chromatographic separations. In this study, excellent selectivity, peak shape, and repeatability were achieved by combining achiral and chiral narrow-bore columns (2.1 mm × 100 mm and 2.1 mm × 150 mm, sub-2 and 3 μm) in the first dimension with 4.6 mm × 30 mm and 4.6 mm × 50 mm columns packed with highly efficient chiral selectors (sub-2 μm fully porous and 2.7 μm fused-core particles) in the second dimension. Multiple achiral × chiral and chiral × chiral 2D-LC examples (single and multiple heart-cutting, high-resolution sampling, and comprehensive) using ultrafast chiral chromatography in the second dimension are successfully applied to the separation and analysis of complex mixtures of closely related pharmaceuticals and synthetic intermediates, including chiral and achiral drugs and metabolites, constitutional isomers, stereoisomers, and organohalogenated species.

Keywords: Chiral, Chromatography, HPLC Columns, Separation Sciences

Abstract Text

Co-Author(s)  Chandan L. Barhate, Christopher Welch, Daniel W. Armstrong

Application Code: Pharmaceutical

Methodology Code: Separation Sciences
Overview of Recent Developments in Ultrafast Chromatography: From Theory to Applications

Critical Contribution of Column Frits to the Separation Performance in High Throughput Liquid Chromatography

The speed of high-throughput gradient liquid chromatography (HT-GLC) is determined by various experimental parameters including injection cycle time (auto-sampler speed), gradient delay, column length, flow rate, temperature, and particle size. The separation power of HT-GLC is controlled by the nature of the stationary phase (particle size primarily) and by the various sources of extra-column band broadening including sample volume, pre- and post-column connectors, and detection system (UV, MS, etc...). Additionally and often unsuspected, most chromatographers ignore how critical the contribution of the column frits to HT-LC performance can be when using extremely short columns.

The purpose of this presentation is to reveal quantitatively the amount of sample dispersion caused by standard frits in 2.1 mm i.d. columns and its impact on HT-GLC performance. Direct and indirect measurement methods are presented and reveal that each frit generate sample dispersion in the range from 0.1 to 0.5 [micro]L2 over a range of flow rate from 0.1 to 0.5 mL/min. Peak capacity per unit time are then compared in the presence and absence of outlet frit for various column lengths (from 0.5 to 3 cm) and the separation of small molecules by using 1.6 [micro]m core-shell particles for ultra-fast liquid chromatography. Results are discussed in terms of designing proper frits for the next generation of HT-LC columns.

Keywords: HPLC Columns, Liquid Chromatography, Pharmaceutical
Application Code: High-Throughput Chemical Analysis
Methodology Code: Liquid Chromatography
Chromatographic analysis of chiral compounds by conventional HPLC can be challenging for compounds with multiple chiral centers as the number of potential stereoisomers increases with increase in number of chiral centers. 2D-LC involving reverse phase in both dimensions can be been used for simultaneous achiral-chiral analysis, however, majority of chiral compounds can not be resolved by reverse phase HPLC requiring normal phase separation. Coupling dissimilar techniques is extremely challenging if not impossible as the sampling criteria and operation modes differ with increase in dissimilarities between dimensions. A fully automated 2D LC-SFC was designed coupling RPLC in the primary and SFC in the secondary enabling simultaneous achiral-chiral analysis of pharmaceutical compounds in a single chromatographic run. The heart of the 2D LC-SFC system is the novel, automated interface enabling effective trapping, focusing, transfer and analysis of primary column eluent in a normal phase SFC system in the secondary dimension. The primary column resolves the potential impurities including the diastereomeric pairs from the main component whereas the secondary chiral SFC column resolves the stereoisomers. Multiple components of interest can be trapped in the interface for subsequent analysis in the secondary dimension. Also, the fractions can be assessed on an array of secondary columns enhancing efficiency. The presentation will cover the design and application 2D LC-SFC system in the analysis of compounds with multiple chiral centers. The results of this study demonstrated the comparability of secondary SFC separation to conventional 1D SFC separation validating the proof of concept. The LOD, LOQ, linearity and reproducibility of 2D LC-SFC will also be presented. With the increase in new modalities, 2D LC-SFC is likely to play a pivotal role in pharmaceutical industry. Additionally, 2D LC-SFC is truly orthogonal.

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Abstract Text
Overview of Recent Developments in Ultrafast Chromatography: From Theory to Applications

Putting Fast Chromatography to Work: Recent Applications in High Throughput Analysis and MISER Kinetic Profiling of Organic Reactions

The speed of chromatographic separations continues to evolve, with baseline resolution of many mixtures being possible in only a few seconds. With the advent of newer, high speed autosamplers and techniques such as MISER (Multiple Injections in a Single Experimental Run) researchers are now well positioned to put fast chromatographic separations to work in everyday research and development projects. We present several recent examples showcasing applications of fast chromatography in high throughput screening for pharmaceutical process research, rapid chromatographic enantioseparation and online analysis and kinetic profiling of organic reactions.

Relevant Publications:
J. Chromatogr. A, 1499, 211-216, 2017
Tetrahedron, 73, 5048-505, 2017

Abstract Text

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Keywords: Chiral Separations, High Throughput Chemical Analysis, Liquid Chromatography/Mass Spectroscopy,
Application Code: Pharmaceutical
Methodology Code: Liquid Chromatography/Mass Spectrometry
Overview of Recent Developments in Ultrafast Chromatography: From Theory to Applications

Microchip Electrophoresis Devices with Monolithically Integrated Electrospray Emitters for Rapid Analysis of Biological Materials

We have pioneered the development of a sensitive, stable, and efficient microchip electrospray interface that enables the integration of MS detection with rapid and highly efficient microfluidic separation methods. The separative performance of these devices is near the theoretical diffusional limit for cationic species. This performance is achieved through the use of novel surface modification strategies that result in highly homogeneous surface characteristics. These devices yield electrospray ionization (ESI) sensitivity commensurate with commercial nanoESI emitters without sacrificing separative performance. Compared to CE-MS performed using fused silica capillaries, microchip CE-MS can achieve greater separation efficiency in shorter analysis times as the integrated injection and ESI functional elements greatly reduce extra-column band broadening. Microchip CE-ESI-MS has been used for challenging applications such as the characterization of intact biopharmaceuticals and antibody-drug conjugates, where achieving optimal separation efficiency is crucial for the success of the analysis. Moreover, light and heavy chain analysis and peptide mapping can also be performed rapidly with high coverage. These separations are generally completed in less than three minutes and most samples require minimal sample preparation. We have used the same technology to address bioanalytical assays such as metabolomic and clinical assays. Our group has also developed the technique of high-pressure mass spectrometry (HPMS) that enables very compact mass spectrometry platforms. As a natural progression of development we have also integrated these two miniaturized platforms. Various elements of the above will be described in this presentation.

Keywords: Bioanalytical, Biopharmaceutical, Capillary Electrophoresis, Pharmaceutical
Application Code: Pharmaceutical
Methodology Code: Microfluidics/Lab-on-a-Chip
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<th>Session Title</th>
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<td>Abstract Title</td>
<td>Chromatographic Separations at the Sensor Timescale: Trials, Tribulations, and Triumphs</td>
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<tr>
<td>Primary Author</td>
<td>M Farooq Wahab, University of Texas at Arlington</td>
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<tr>
<td>Co-Author(s)</td>
<td>Daniel W. Armstrong</td>
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**Abstract Text**

For more than four decades, separation scientists have directed their efforts in designing new surface chemistries to separate difficult chiral or achiral separations in various chromatographic modes. A quick look at the recent literature will reveal that high-throughput screening is gaining an exponential momentum, where difficult separations in 1-2 minutes are becoming routine. In contrast, a typical sensor takes a few seconds to respond to a single analyte in a simple matrix. Chromatography can handle very complex mixtures and difficult analytes. We directed our efforts to combine the best of the world of sensor's speed and resolution of chromatography. The idea of sub-second separations for chiral and achiral separations is becoming true; with potential applications in two dimensional LC and fast screening.

A number of challenges and their solutions will be discussed while demonstrating the utility of sub-second hyperfast separations. The first and foremost challenge after synthesis is to pack 0.5 cm to 1 cm long stable columns with very small core-shell or fully porous particles (< 2 µm). The chromatograph needs to be optimized for reducing pre-and post-column band broadening to the best possible extent but these dispersion effects cannot be eliminated. Fourier deconvolution can eliminate such effects. Additionally, the power of peak treatment procedures such as power law and new derivative based method for peak sharpening will be discussed.

**Keywords:** Chiral Separations, Liquid Chromatography, Separation Sciences, SFC

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

**Methodology Code:** Liquid Chromatography
The electrochemical properties of materials are of great interest in the area of heterogeneous catalysis. For these systems, the surface composition and structure of a material greatly influence the electrochemical behavior. Developing an understanding of these structure-property relationships via studies combining electrochemical and physical methods allows for the design and synthesis of improved materials for a variety of applications. Specifically in this talk, Fe-based non-precious metal catalysts for the oxygen reduction reaction will be discussed.

Non-precious metal catalysts have been highly sought after to replace the Pt-based catalysts currently employed in fuel cells to facilitate the oxygen reduction reaction. After over 50 years of research, non-precious metal catalysts are now approaching the activity of Pt but a detailed understanding of the active site remains elusive. We have subjected a prepared catalyst to different oxidizing treatments in order to modify the catalyst structure and modulate the catalytic activity. Using several complementary methods to study the structure of the prepared and treated catalysts, we reveal the chemical species that lead to high catalytic activity and provide crucial direction for the future development of new catalysts with improved performance.

**Keywords:** Electrochemistry, Electrode Surfaces, Materials Characterization, Surface Analysis

**Application Code:** Material Science

**Methodology Code:** Electrochemistry
Use of electrocatalysts for small-molecule transformations has gained widespread interest among the chemistry community, particularly for environmental and energy relevant transformations such as oxygen reduction and evolution, and CO$_2$ reduction. Solid-state electrocatalytic systems typically operate with high levels of activity but suffer from poor selectivity and/or tunability. Alternatively, molecular electrocatalysts with tunable ligands are often capable of high selectivity for a single product but operate with comparatively lower activity than their solid-state counterparts. The goal of this work is to develop 3-D architectures formed of discrete molecular catalysts that will operate with the specificity of molecular catalysts while maintaining the activity of traditional solid-state systems. This will be done by developing a method for layer-by-layer growth of discrete molecular catalyst films using sequential Cu(I)-Catalyzed Azide-Alkyne Cycloaddition reactions. By increasing the number of catalyst layers, we can increase the activity per unit area of our electrode while maintaining the specificity of the discrete molecular catalysts. Additionally, the films can theoretically be formed using any complex with a diethynyl functionality making them highly modular. As a proof of concept, we have synthesized a series of electrodes modified with single, double, and triple layer films of copper diethynylphenanthroline a known electrocatalyst for oxygen reduction. We have found that both the concentration of copper on the surface and the activity level of the electrodes increases as a function of the number of layers.
While electrified transition metal surfaces mediate the synthesis of carbonaceous fuels from CO2, these processes suffer efficiency losses due to the multitude of products accessible over a narrow potential range. Selective product formation requires knowledge and control over various branch points in the reaction pathway. In this talk, we will discuss two specific branch points: (1) the requirements for selective activation of CO2 over H+ to form the two-electron reduced CO product; and (2) the requirements for accumulation of surface-bound CO species that can be reduced to higher order products beyond CO. Using model Au and Cu catalysts, we uncover mechanistic insights into these branch points. First, we identify the differential proton coupling requirements for CO2 versus H+ activation on Au surfaces. Second, we elucidate and compare the differential CO adsorption dynamics on Au and Cu surfaces using in-situ spectroscopic studies. While on Au surfaces, we observe a low population of surface-bound CO that readily dissociate from the surface, labile CO species accumulate on the Cu surface providing a pool of reactant primed for further reduction to higher order products.
Electrodialysis is a commercial technique used for water desalination, acid recovery, and neutralization of water streams. The efficiency of electrodialysis suffers from energy loss due to the large overpotential needed to drive water electrolysis and unintended electrochemical side reactions. Many electrochemical techniques use passive ion-exchange membranes, such as Nafion®, which provide the ability for selective migration of ions in between chambers. If these ion exchange membranes could be adapted to drive photo-induced charge separation of a proton and a hydroxide water could be dialyzed directly, avoiding electrolysis of water. This new process could provide up to an 80% increase in energy efficiency. Previously reported photovoltaic cation exchange systems (cPFSA) offered a promising starting point for these systems but couldn’t stop back diffusion of counter ions across the membrane and lacked the photovoltages necessary to desalinated brackish water or seawater.

Considerable progress has been made towards a workable direct solar to water desalination membrane by redesigning the device morphology from a monopolar structure to pn-type structure of photo-sensitized bipolar membrane (PSBM) using several commercial anion exchange membranes. This redesign marks a significant improvement of the device performance by limiting co-ion diffusion across the membrane and increased the photovoltage from 1 mV in cPFSA to greater than 130 mV in the pn structure. However, poor contact between the ion-exchange materials resulted in the reduction of the photocurrent from 100 µA/cm² to 10 µA/cm² and unideal rectification behavior. Analogies to traditional pn solar cells and PSBM were made by measuring spectral response, fluence dependence, and time resolved microwave conductivity. PSBM marks a cornerstone in the development of a functional direct solar to water desalination which requires 180 mV to desalinate brackish water to a potable concentration.

Abstract Text

Keywords: Electrochemistry, Membrane, Semiconductor, Water
Application Code: Material Science
Methodology Code: Electrochemistry
Ion channels are nature’s nanopores that can be exploited as nanoscale biosensors by monitoring changes in an ion current that flows through the channel(s). In the presence of a target, the measured ion current can change; typically due to the target molecule translocating into or through a channel, or binding to the membrane protein that may open or close the channel. Specific molecule binding is particularly attractive for biosensor applications, in which the concentration of a single analyte is required. Different membrane proteins that bind to specific targets, including previously unattainable molecules, can be incorporated into a lipid bilayer to enable molecule-specific nanoscale biosensors with single molecule sensitivity.

Another strategy for specific biomolecule detection is electrochemical aptamer-based sensors. Electrochemical aptamer-based (E-AB) sensors are short DNA/RNA molecule-modified sensors that bind specifically and reversibly to a target molecule, resulting in a change in signal response. Typically E-AB sensors are large (often on the mm scale), and this will limit their use for probing local special information, in particular for biological samples, approaching the single cell level. Ultimately, both these biosensor methods can be applied to study the cellular environment to understand communication at the single cell level.
### Abstract Text

Implantable, continuous glucose biosensors are an essential tool for diabetic patients in monitoring and controlling their disease. However, commercially available continuous glucose biosensors are only FDA-approved for up to 7 days of use due to significantly diminished sensitivity, caused by a host-mounted foreign body response (FBR). Nitric oxide (NO) is an endogenously produced gas molecule that regulates many physiological processes, including the inflammatory response. Previous results suggest that low levels of exogenously supplied NO can reduce the FBR, and thus improve performance and extend the lifetime of implanted glucose biosensors. In this research, NO-releasing silica nanoparticles in polyurethane sensor membranes were demonstrated to be capable of releasing NO for up to 30 days. By varying the hydrophobicity and concentration of the polyurethane, as well as the concentration of the particles, suitable sensor performance was achieved in vitro. In particular, the sensitivity and linear dynamic range of the sensors showed dependence on these sensor fabrication parameters. Furthermore, NO-releasing sensor membranes were implanted into pigs for an in vivo evaluation of sensor performance and histological inflammatory response as a function of continuous NO release.

**Keywords:** Bioanalytical, Biosensors, Electrochemistry, Materials Characterization

**Application Code:** Bioanalytical

**Methodology Code:** Sensors
Electrochemical aptamer-based (E-AB) sensors rely on target-binding-induced conformational change and are commonly used to rapidly detect targets in complex samples, making them favorable for on-site drug detection. Sensitive detection of targets using E-AB sensors requires optimization of aptamers to achieve a large target-induced conformational change. Specifically, optimization involves aptamer truncation and sequence engineering which is often time-consuming and laborious. To date, there are no universal strategies to rapidly engineer the sequence of isolated aptamers for use in E-AB sensors. We here utilize exonucleases to engineer a structure-switching MDPV-binding aptamer (MDPV-46) for the electrochemical detection of synthetic cathinones in seized substances. We performed Exo III digestion of MDPV-46 in the presence and absence of MDPV. MDPV-46 has a three-way junction binding domain with a duplexed, blunt-ended 3’ terminus. Without MDPV, Exo III catalyzed the 3’-to-5’ digestion of MDPV-46 to form 33-nt and 39-nt single-stranded products. With MDPV, however, Exo III digestion was halted five bases prior to the binding domain to form a major digestion product (43-nt) with double-stranded structure. Isothermal titration calorimetry experiments demonstrated that the binding affinity of the 43-nt product is very close to that of MDPV-46. The 43-nt product generated from Exo III digestion is directly employed for electrochemical detection of MDPV. The E-AB sensor demonstrates high sensitivity for MDPV with a linear range of 0 to 20 µM and a detection limit of 1 µM. The sensor also displays high specificity for 10 other synthetic cathinones. In contrast, no signal is observed upon the introduction of common cutting agents and controlled substances. We believe our strategy can serve as a universal method for easily engineering structure-switching aptamers from newly isolated aptamers for use in E-AB sensors.

Keywords: Bioanalytical, Biosensors, Electrochemistry, Enzyme Assays
Application Code: Bioanalytical
Methodology Code: Electrochemistry
Electrochemiluminescence (ECL) refers to the photoemission activated by electrode driven electron transfer (ET) reactions. Chemiluminescence (CL) is not limited by the heterogeneous ET but also results from homogenous ET processes. Redox activity of reagents is essential in the generation of ECL or CL that finds wide applications in sensing, assay among many other applications. Gold nanoclusters stabilized by a monolayer of ligands are well-known to exhibit rich electrochemical and optical properties. Here, basics and exciting potentials of Au nanocluster ECL and CL in representative metal ion and ROS sensing applications will be discussed. ECL in the near infrared spectrum window, highly advantageous in sensing with reduced interferences, is generated under ambient atmosphere conditions and physiological pH ranges. Two routes will be introduced to drastically enhance the ECL through the interplay between the electronic structures of Au nanoclusters and the employed molecules/orbitals. The first is adopting common chelators such as EDTA and HEPES in a conventional free diffusion enhancement pathway for generalizable and broader applications. The second is to covalently attached molecules with accessible and matching redox properties as part of ligand monolayers. The corresponding ECL generation pathway is greatly simplified by such intra-nanocluster charge transfer mechanism. Impacts of metal ions and pH reveal interesting charge and/or energy transfer associated with the highly reactive intermediate species that is under further investigation.

**Keywords:** Biosensors, Electrochemistry, Luminescence, Near Infrared

**Application Code:** Nanotechnology

**Methodology Code:** Electrochemistry
VOC’s represent a common class of environmental pollutants with known ecotoxicological and human health effects. As such, solid phase microextraction (SPME) methods have been used as environmentally friendly alternatives to traditional solvent based sample preparation techniques which create additional VOC waste themselves. However, most traditional SPME sorbents, typically hydrophobic in nature, and their corresponding low volume, small surface-area fibre morphologies may not offer sufficiently sensitivity required for the ultra-trace determination of highly toxic VOC’s, particularly those that are polar in nature such as disinfection by-products.

With these limitations in mind, the proposed work presents a highly sensitive HLB-PDMS thin film microextraction device for the balanced determination of VOC compounds of varying polarity. In addition to exhibiting a 50 fold increase in sensitive when compared to a 65 um DVB/PDMS SPME fibre, these membranes extracted double the amount of McReynolds standards Vs. a move comparable DVB/PDMS TF-SPME device of identical size. The McReynolds compounds tested included benzene, 2-pentanone, 1-nitropropane, pyridine, 1-pentanol and octane. Furthermore, Inter-membrane extraction efficiencies for these compounds were determined reproducible at 95% confidence for all 4 of the coating chemistries tested including the DVB/PDMS membranes, and those prepared with 3 different HLB compositions. Further method reliability was established by confirming that, once extracted, the McReynolds standards were stable on the HLB/PDMS membranes stored in the thermal desorption tubes on the autosampler rack for at least 120 hours for 5 of the 6 standards and only 24 hours for pyridine at 95% confidence. Finally, a real-world proof of concept application determining chlorination by-products from a private hottub was performed, successfully identifying, 2-chloroethylamine 3-chloro-1-propanamine, and dichloroacetonitrile with %RSD’s less than 10%.

Keywords: Environmental Analysis, GC-MS, SPME, Volatile Organic Compounds
Application Code: Environmental
Methodology Code: Gas Chromatography/Mass Spectrometry
Smallmouth bass Micropterus dolomieu in the Susquehanna River Basin have been a species of interest for over a decade due to continued fish health concerns. Collaborative research efforts have identified a wide range of disease problems as well as potential risk factors. Nevertheless, there is still no understanding of organic contaminants in the environment and in fish tissues that are related to observed lesions and functional health impairments in the affected fish. So far, studies of cause-effect-relationships have been utilized by targeted analysis that excludes many contaminants, which may contribute directly or indirectly to resident organism health impacts. Therefore, prior investigations have been inconclusive regarding the correlation between disease and exposure. The presented study was aimed at developing a more inclusive, discovery-based, analytical approach that can achieve a reasonable sample throughput. Therefore, sample preparation that allows for high throughput of biological tissues was combined with highly sophisticated two-dimensional gas chromatography coupled to time-of-flight mass spectrometry (GC×GC-TOFMS). The method was applied to the non-targeted analysis of organic contaminants in young of the year smallmouth bass from the Susquehanna River Basin, which may provide insight into exposures to contaminants at a highly sensitive life stage.

The research was supported by the Pennsylvania Sea Grant.

Keywords: Contamination, Environmental/Biological Samples, Gas Chromatography/Mass Spectrometry, Methodology Code: Environmental

Methodology Code: Gas Chromatography/Mass Spectrometry
The ability to improve analytical detection limits is advantageous. When instrument parameters ensure improved detection limits, a smaller sample size may be used for extraction and method detection limit requirements are still attained. This has many benefits including using less solvent for extraction which is safer for the environment and reduces costs.

This presentation demonstrates how enhanced injector and GC/MS technologies can reduce laboratory costs and enhance laboratory throughput by enabling the collection of smaller sample volumes and still be able to meet and/or exceed EPA criteria.

If a significantly smaller sample size is being used, less extraction solvent will be used and less storage space is needed for the same number of samples. In most instances, the concentration step can be avoided providing additional cost savings for the laboratory and increased laboratory throughput.

The use of large volume injections and simultaneous full scan/single ion monitoring acquisition to enhance precision at low concentrations and to further improve detection limits will also be discussed using the analysis of polynuclear aromatic hydrocarbons (PAHs) and pesticides.

In addition, a micro channel switching device has been employed to backflush most unwanted components from the column and injector increasing system uptime and sample throughput.
The need for accurate monitoring of chemicals in air using US EPA Method TO15 continues to grow in importance as the comprehension of their impact on human health evolves. Over the past several years, laboratories running Method TO15 have seen a sharp increase in the number of soil gas samples that must be analyzed with this method. Soil gas concentrations can climb to levels a million times higher than that of ambient air, creating a challenge for analytical systems to avoid contamination, which in turn can cause a loss in laboratory productivity. A means to provide pre-screening and a reduction in carryover is needed. A new canister preconcentrator and autosampler design is presented which increases method accuracy while improving immunity to system contamination, all without the use of liquid nitrogen or electronic cooling previously required to perform TO15 analysis. Elimination of cryogen expenses creates substantial savings for laboratories. This revolutionary trapping technology called Multi-Capillary Column Trapping System (MCCTS) has been developed using open tubular capillary columns in series with increasing strength to trap complex air samples containing compounds over a wide range of volatilities. The trap design shows less susceptibility to contamination when exposed to high concentration samples, reducing laboratory downtime. A new canister autosampler design uses a single heated inlet to connect to up to 48 canisters only during the time of sample extraction, with flushing of the line after sample preconcentration. This dramatically reduces system carryover relative to rotary valve based autosamplers. A pre-screening feature is also built into the autosampler, avoiding all contact with the preconcentrator traps, eliminating the potential for system contamination. Full TO15 validation is demonstrated including blank levels immediately after running higher concentration samples containing BTEX, PCE, and TCE often found in soil gas at high PPM levels.

Keywords: Environmental/Air, Environmental/Soils, Trace Analysis, Volatile Organic Compounds
Application Code: Environmental
Methodology Code: Gas Chromatography/Mass Spectrometry
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<td>Abstract Title</td>
<td><strong>Combining GCxGC with High Resolution Mass Spectrometry for Accurate Identification of Analytes in a Complex Environmental Sample</strong></td>
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<tr>
<td>Primary Author</td>
<td>Scott Pugh</td>
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<tr>
<td>Co-Author(s)</td>
<td>Georgy Tikhonov, Slava Artaev</td>
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<tr>
<td>Date:</td>
<td>Wednesday, February 28, 2018</td>
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**Abstract Text**

Gas chromatography coupled with mass spectrometry provides some of the best analytical tools which combine selectivity, sensitivity, reliability, and information capacity for both targeted and non-targeted methods of environmental analysis. The GC-MS identification of known compounds of interest and the structural elucidation of unknown compounds becomes considerably more reliable if accompanied by accurate mass measurements while using High Resolution Mass Spectrometry (HRMS). When analyzing real-life samples in a complex matrix, a large number of analytes of interest with a wide range of concentrations are likely present. Consequently, a significant increase in chromatographic peak capacity is required which can be realized by the use of comprehensive GCxGC. An ultra-high resolution time-of-flight mass spectrometer with enhanced sensitivity was used to analyze a variety of environmental samples using various analytical techniques for accurate identification of the analytes in the samples. Examples will be shown of how to use HRMS combined with GCxGC to have better confidence in finding and identifying analytes in very complex samples.

**Keywords:** Environmental Analysis, GC-MS, Mass Spectrometry, Time of Flight MS

**Application Code:** Environmental

**Methodology Code:** Gas Chromatography/Mass Spectrometry
Polychlorinated dibenzo-p-dioxins (PCDD) and dibenzofurans (PCDF) have been important compounds to analytical chemists for decades, with the first publications describing analytical techniques for their identification appearing in the 1970s. The international scientific community has acknowledged that these compounds are known to be detrimental to and accumulate in the environment and animals. The combustion processes that form dioxins and furans often form other halogenated compounds, such as polychlorinated biphenyls (PCBs), which can be potentially toxic as well. The mixture of these compounds in environmental samples poses a daunting analytical challenge for chromatographers. Many of the analytes of interest have been extensively characterized on specific columns. This has been achieved using QSRR models, isotope dilution, and retention indices. Different characterization techniques are often applied to different compound classes, thus making comparison between compound classes difficult. A retention system based off Kovats and Lee retention indices is proposed here for PCBs and dioxins as a means to identify coelutions and congeners on a variety of columns. This data can also be used to determine optimal column pairings for two-dimensional GC analyses.
Historically targeted analysis has been the primary route to evaluate complex environmental samples. This constrained testing, while effective has often missed emerging or unexpected compounds within samples. Recent improvements in detection and data processing capabilities of various systems have allowed scientists to more fully evaluate these same samples using non-targeted (NT) techniques. As a result, the EPA is currently conducting a multiple lab, multiple platform evaluation for non-targeted analysis methods in samples designed to mimic the environmental exposome. The project contains two initial phases, first a blinded study is conducted and reported. In phase two the individual standard component lists are provided and the evaluation revised as necessary. Each blind standard is reported to contain between 100-400 spiked analytes with potential for more due to contaminants, intra-sample degradation or reaction product formation. This presentation describes the systematic logic used for identification of the unknowns, its results and the lessons learned from the process as it applied to the first round of ten, blinded ENTACT samples for a single platform. The platform used was a comprehensive GCxGC gas chromatograph coupled with a high resolution accurate mass (HRAM) time of flight mass spectrometer (TOF-MS) in both electron ionization (EI) & chemical ionization (CI) modes. Deconvolved spectra were matched to existing commercial MS libraries and screened based on the peak’s retention index value, molecular ion mass accuracy and fragment ion formula fidelity. Questions addressed will be: (i) what percentage of each sample was correctly identified, (ii) what instrumentation characteristics contributed most significantly to the identification and (iii) what impurities, reaction products and degradation products were identified.

**Abstract Text**

**Keywords:** Environmental, Environmental Analysis, GC-MS, Time of Flight MS

**Application Code:** Environmental

**Methodology Code:** Gas Chromatography/Mass Spectrometry
Environmental forensics addresses scientific questions, which typically involve the analysis of complex matrices in limited amount of samples. Commonly these questions are approached using targeted, analytical techniques. However, targeted approaches tend to disregard the main part on information in the analyzed sample.

With the advent of cutting edge separation techniques, e.g. GCxGC, untargeted analysis gains more and more importance. With their high peak capacities these techniques are therefore perfectly suited for the analysis of complex matrices. Due to the narrow peak widths from cryomodulation high data acquisition frequencies are prerequisites for a compatible detection system. Therefore, only a few detection systems have been routinely utilized, e.g. TOF-MS or FID. These instruments provide a solid work. Nevertheless, additional types of mass spectrometers may allow for an overall increase in data quality.

Coupling of nominal-mass MS/MS detection to comprehensive separation provides benefits for certain analyses, especially in difficult matrices where a wide range of compounds are being measured. Especially when sample mass or volume is limited and the range of measured compounds is wide, GCxGC coupled to MS/MS may provide for enhanced performance and data analyses relative to the other possible choices.

This presentation will address the applicability of GCxGC-MS/MS to environmental forensic questions. It will show recent instrumental modifications to a commercial system which have allowed for continued increases in sensitivity. Using multiple reaction monitoring (MRM) detection, attogram levels of detection are possible, while retaining the GCxGC separation power and the ability to monitor for a range of compounds.

This will be discussed as a possible path towards the ultimate in detectability; maintaining the peak capacity and resolving power of the GCxGC technique at the lowest levels of detection currently possible with MRM.
Nanoparticles (NPs) have been reported as efficient matrices in MALDI-MS analysis of small molecules due to their low matrix background, homogeneous application, and high laser absorptivity. Recently our group has screened 13 NPs (metal oxides, carbon-based, and metals) for their efficiency as matrices for small molecule analysis (Anal. Chem. 2016). Metal NPs tend to aggregate in solution which limits their usefulness as matrices. To avoid this, we adopted physical vapor deposition as a means to create metal NPs [i]in situ[/i] and six metals deposited via sputter coating were tested. Combining both NP screening results, as well as selected organic matrices, a turkey cecum extract was tested to determine the optimum set of matrices for the widest metabolite coverage. Five matrices, Fe[sub]3[/sub]O[sub]4[/sub] NPs, CHCA, and Au in positive mode, and Cu and Ag in negative mode, offered the most comprehensive coverage and were used to create a pre-spotted NP microarray for high-throughput metabolomics analysis of turkey microbiomes. Our optimized NP microarrays were then used to compare cecal content extracts of non-treated or antibiotic-treated (therapeutic or sub-therapeutic doses) turkeys using a multiplex data acquisition to obtain MS/MS data in addition to high resolution MS. For example, with Ag, 456 unique features were found, and of those 85 are common to all three treatments, but 262 are found only in one sample type. With this high-throughput approach, we will be able to analyze 288 samples in a day, allowing the study of 240 samples collected across 8 time points in about 4 days. Also discussed will be chemical modifications of these complex microbiome samples to selectively enhance ion signals of particular classes of compounds. The data was collected using a MALDI-linear ion trap-Orbitrap MS and analyzed using MSiReader and Xcalibur.

This work is supported by the United States Department of Agriculture-National Institute of Food and Agriculture (USDA-NIFA).

Keywords: Biological Samples, Mass Spectrometry, Metabolomics, Metabonomics
Application Code: High-Throughput Chemical Analysis
Methodology Code: Mass Spectrometry
A new generation of 1.9 μm superficially porous particles are utilized to develop ultrahigh performance chiral and HILIC stationary phases to produce chromatographic separations on the timescale of typical sensors. Optimal column geometries for sub-second separations were determined and efficiencies as high as 285,000 N/m were achieved in 1 x 0.3 cm i.d. columns. These short, ultrahigh efficiency columns produced baseline resolved, sub-second achiral separations of structurally and functionally related analytes such as nucleosides, plant hormones, and salicylic acid derivatives. Sub-second chiral separations are also achieved for biologically active analytes. Removal of extra-column band broadening from chromatograms with multiple analytes is achieved using Fourier Transform deconvolution. A new, derivative based resolution enhancement method is developed to improve sub-second separation of species. Advantages of the new approach include retaining of peak properties such as retention time and peak area. Using the ultrahigh performance phases, an exemplary sub-second separation of 10 analytes was accomplished and further made baseline with the Fourier Transform deconvolution as a proof-of-concept of the promising potential of sub-second separations in ultrahigh throughput separations.

Keywords: Chiral Separations, High Throughput Chemical Analysis, HPLC, HPLC Columns
Application Code: High-Throughput Chemical Analysis
Methodology Code: Liquid Chromatography
Residual solvents or monomers in packaging materials pose a contamination risk for the pharmaceutical or food products that they will contain. Rapid analysis enables wider testing and therefore improved quality control. Selected Ion Flow Tube Mass Spectrometry (SIFT-MS) enables direct, real-time monitoring of volatile compounds and therefore facilitates better quality control. Unlike traditional chromatography-based techniques, there is no requirement to separate compounds prior to analysis. This leads to a number of benefits. Not only can challenging compounds, such as formaldehyde, ammonia and small sulfur compounds, be analyzed without derivatization or pre-concentration, but these analyses can be completed in seconds. By coupling advanced autosampler technology to SIFT-MS instruments, analyses can be entirely automated, fully realizing the potential of the fast analytical measurements. Further, by utilizing the autosampler’s advanced software features, techniques that are cumbersome with chromatography can be turned into practical, economic methodologies, in some cases realizing a ten-fold increase in throughput.

In this paper, a range of automated headspace techniques will be demonstrated, including rapid headspace method optimization and multiple headspace extraction (MHE) methods. These and other high-throughput SIFT-MS packaging applications will be illustrated using residual solvent and monomer analysis from materials such as polyacetal, polypropylene, and polystyrene.

Reference:

Keywords: Chemical Ionization MS, Headspace, Pharmaceutical, Volatile Organic Compounds
Application Code: High-Throughput Chemical Analysis
Methodology Code: Mass Spectrometry
High Throughput Chemical Analysis

Fast GC-MS Analysis – The Way to Real Time Analysis

Typical GC-MS analysis takes 30-40 minutes and often requires lengthy sample preparation. We developed three technologies that facilitate significantly reduced GC-MS analysis time. A) GC-MS with Cold EI which 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 combined use of short column with flow programming enables universal drugs of abuse screening with two minutes chromatography time and three minutes full analysis cycle time. Unique to Cold EI is that its ion source response is ultra-fast and independent on the column flow rate plus the enhanced molecular ion selectivity offset the minor loss of GC separation in fast GC-MS analyses. B) We developed a unique low thermal mass Fast GC that is based on the use of short standard columns in a low thermal mass tube heater. It enables sub one minute full analysis cycle time with a limited factor of 4 only loss of GC separation and it can be coupled with both GC-MS with standard EI and Cold EI. C) We also developed an Open Probe Fast GC-MS for obtaining real time analysis with separation including the elimination of sample preparation. Thus, Open Probe Fast GC-MS serves as a superior alternative to the various ambient ionization methods while adding ultra-fast GC separation and NIST library identification capabilities and compatibility with the low cost MS of GC-MS

Keywords: Gas Chromatography, Gas Chromatography/Mass Spectrometry, GC-MS, High Throughput Chemical Analysis
Application Code: High-Throughput Chemical Analysis
Methodology Code: Gas Chromatography/Mass Spectrometry
Nanoflow liquid chromatography has become a valuable analytical tool for on-site detection and bioanalysis, for which considerable attention has focused on miniaturization of the column and instrumentation. The “Achilles heel” of the nanoflow LC system is the frit technology used to contain the packing materials in the column. Column frits must restrain movement of the particles without adding any mobile phase flow restriction or interaction with analytes. They provide the column interfacing to the other components of the system. Superior quality frits are needed to provide highly reproducible separations especially for on column detection to minimize extra-column band broadening, which becomes more difficult to accomplish as the column diameter decreases. In this work, we introduce a new fritting technology comprising fabrication of high quality and highly reproducible capillary columns for on column detection. The frit is comprised of a short, porous organo-silicon polymer plug made via hydrolyzing octadecyltrimethoxysilane by thermal initiation in the presence of specific organic solvent porogens, acidic catalyst and deactivating reagent. A statistical model was developed for optimizing the relative percentages of the reagents and processing conditions based on scientific principles, which aided in simplifying the development process. The new frit technology allows the use of a wide range of silica-based stationary phase materials with different particle sizes (3-5 microns) in capillaries of differing lengths (5–25 cm) and internal diameters (< 150 microns). Single monomer frits provide advantages such as good mechanical stability, high reproducibility and simple fabrication. The developed fritting technology was evaluated by separations of samples containing compounds of different polarities for fast and reliable preparation of stable, high performance capillary LC columns.

Keywords: Capillary LC, HPLC Columns, Instrumentation, Material Science
Application Code: High-Throughput Chemical Analysis
Methodology Code: Liquid Chromatography
The rapidly advancing fields of pharmaceuticals, pesticide analysis, and food testing have encountered a major challenge in the separation and quantitation of the many highly polar analytes of interest. Adequately retaining and separating small polar molecules with reversed-phase liquid chromatography (RPLC) is often challenging. Alkyl phase LC columns, like C18, are a common starting point for LC method development, but highly polar analytes are poorly retained on these non-polar stationary phases.

Several techniques have been developed to analyze polar compounds: pH adjustment, ion pairing, normal phase chromatography, derivatization, ion chromatography, and hydrophilic interaction chromatography (HILIC). Of these, HILIC has rapidly gained popularity among many chromatographers for its ability to retain and separate polar analytes.

Superficially porous particles are known for their ability to generate high efficiency with low back pressure. This high efficiency is critical to resolving closely eluting peaks, while low back pressure allows for flexibility with LC instrumentation. These distinct advantages make superficially porous particles the ideal platform for developing new HILIC chemistries.

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. New HILIC chemistries on superficially porous particle columns will demonstrate these method considerations along with technical advancements in the area of highly efficient HILIC separations when compared to traditional HILIC stationary phases.

Keywords: Amino Acids, Carbohydrates, Food Science, Pharmaceutical
Application Code: High-Throughput Chemical Analysis
Methodology Code: Liquid Chromatography
Migrating from conventional liquid chromatography (LC) with large columns and milliliter per minute flow rates to nanoflow liquid chromatography with capillary columns and nanoliter or microliter per minute flow rates has provided several advantages and challenges. Advantages of small columns and low flow rates include greatly reduced solvent consumption and waste generation, small sample volumes and ease of interfacing to mass spectrometry. However, most nanoflow systems are not able to perform sequential analyses as quickly as conventional systems because of their dwell volumes. Connecting tubing, mixer volume and transfer lines contribute volume that is insignificant at high flow rates; however, this volume increases the time required for injection and solvent change at low flow rates, such that the overall system throughput is low. For instance, a 20 cm transfer line with an inner diameter of 0.15 mm gives a dwell time of more than two minutes at a flow rate of 1.5 microliters per minute.

We have been developing a nanoflow LC system that is designed to allow rapid, sequential analyses for high throughput. In order to accomplish this, several systemic changes have been made. The injector was moved very close to the head of the column, reducing the time it takes for sample to reach the packed bed. Miniature UV absorption detectors were fabricated to perform on-column detection at less than 1 mm past the end of the column, practically eliminating post-column band broadening. To reduce the time required for solvent change to reach the column, a very low internal volume mixing tee was placed immediately adjacent to the injection valve.

These and other optimizations have allowed sequential runs with less than 1 minute between analyses, while maintaining ease of use and tool-free column switching. In addition, a novel dual sequential column, dual detector arrangement has become possible because of these combined nanoflow optimizations.

Keywords: Capillary LC, HPLC, Instrumentation, Portable Instruments
Application Code: High-Throughput Chemical Analysis
Methodology Code: Liquid Chromatography
As typical for many businesses, laboratories are assessed on their efficiency in terms of operations excellence, costs, quality, and response time by measuring key performance indicators (KPI). KPIs are based on the output at a certain quality per unit time. This output is balanced against related costs. Analytical laboratories must meet these demands for enhanced productivity, as well as demands for use of multiple and diverse chromatography techniques, by analyzing more samples in the same time with the same amount of personnel, equipment and space, while maintaining rigorous quality standards. Highly automated lab equipment contributes the most to increased productivity and allows laboratories to deliver improvements in KPIs.

The Thermo Scientific™ Vanquish™ UHPLC productivity workflows address the productivity demand with a smart combination of unique dual technologies, by building two flow paths into a conventional UHPLC housing. Each flow path consists of a pump, an injection unit, a column and a detector. This Dual LC technology doubles the number of assays in the same time. Similarly, a second pump in a system can also reduce the overall analysis time by up to 60% when used for overlapped offline column wash and re-equilibration in a Tandem LC setup. Another use is the example of a Charged Aerosol Detector combined with a Dual Gradient Pump in an Inverse Gradient setup to quantify unknown compounds without the need for expensive standards. These three examples highlight the benefits of an UHPLC system and show how it maximizes efficiency.

Keywords: Analysis, HPLC, Optimization, Sample Handling/Automation
Application Code: Other
Methodology Code: Liquid Chromatography
Detection of Common Antibody Drug Conjugate Solvents via Ionic Compound Partitioning by Headspace Gas Chromatography

Antibody Drug Conjugates (ADCs) are complex hybrid molecules comprised of a monoclonal antibody connected to a small molecule drug through a linker. The key step in the production of ADCs is bringing together the protein in an aqueous buffer with a hydrophobic small molecule in order to achieve conjugation of the molecules. This step involves dissolving the small molecule portion of the compound in an aqueous miscible organic solvent. The determination of residual solvents in pharmaceuticals is of significant importance in to protect patient safety and ensure an efficacious drug. Headspace gas chromatography (GC) is the most widely used tool for quantification of residual solvents for small molecule APIs but is not widely used for the analysis of protein containing samples. Detection of residual solvents in headspace injections was explored using various conditions in order to detect the solvents in an ADC drug product sample. Among the most commonly used conjugation solvents are N,N-dimethylacetamide (DMA), N,N-dimethylformamide (DMF), dimethyl sulfoxide (DMSO), ethylene glycol (EG), and propylene glycol (PG). However, EG and PG do not partition into the headspace efficiently in the ADC drug product samples that contain large amount of water. In order to address this limitation several ionic liquids and other ionic compounds were screened with the ADC samples to see if they could improve the portioning of the key solvents EG and PG. After identifying the best-performing ionic compound, different columns were screened in order to develop and optimize a suitable headspace GC method capable of detecting and resolving our solvents of interest. Out of all the ionic compounds investigated, 1-butyl-2,3-dimethylimidazolium was the only one found to partition EG and PG efficiently into the gas phase, as well as significantly enhancing the response of DMA, DMF, and DMSO on an optimized headspace GC method.

Keywords: Gas Chromatography, GC, Pharmaceutical, Sample Introduction
Application Code: Pharmaceutical
Methodology Code: Gas Chromatography
Liquid chromatography (LC) systems are routinely used to analyze complex mixtures, for which high separation power is always an important goal. One approach to achieve this is through multi-dimensional LC, which has been available for some time. Compared to one-dimensional LC, two-dimensional LC utilizes two types of molecular interactions in distinctly separate (but coupled) separations to achieve high separation power and large peak capacity. However, multi-dimensional LC requires complicated components (valves, traps, etc.) to enable sample transfer between different separation columns and to control mobile phase flow and composition. We recently found that by placing two different column segments directly in series and using a small, single wavelength light-emitting diode (LED) detector after each column segment, we can achieve improved separations while retaining the ease-of-use of one-dimensional analysis. Using this design with a single injection, retention times from two columns and UV absorbance measurements at different wavelengths can be obtained without a diode-array detector. Thus, a complex mixture can be analyzed using two separation mechanisms, such as two different reversed phases, or a combination of a chiral separation with a reversed phase, in one analysis. The simplicity and effectiveness of this approach makes it an ideal candidate for target analyte detection in a portable nano-flow LC system, where the separation of complex sample mixtures is needed, but mass spectrometry or two-dimensional LC is not practical.
Over the last decade the use of Pharmaceuticals and Personal Care Products (PPCPs) has doubled in the United States. As a result, PPCPs have entered the environment through both human activity and as byproducts from manufacturing, agricultural activities, medical use and veterinarian facilities. This has led in some cases to ppm level concentrations of these compounds in the Great Lakes and significant risks to the ecosystem.

Solid Phase Extraction (SPE) can be used for measuring any of these compounds in water samples. Automation of the sample prep process can result in faster turnaround time of samples, lower costs, and improved quality of the data generated.

During automated SPE runs with PPCPs, cartridges were conditioned with methanol and water and 1 L samples were loaded onto the system. Cartridges were then dried with nitrogen under vacuum, and eluted with methanol/base fraction and formic acid/methanol. Samples were reduced in volume via automated evaporation.

Excellent recoveries were seen between 80-100%. Efficiency of the extraction was increased by the use of nitrogen and vacuum to dry the cartridge and a water free extract that enabled a fast concentration step with no loss of analytes. Direct-to-vial connections eliminated the necessity of sample transfer. After concentration samples were ready for LC/MS analysis. This eliminated human error, saved time and increased efficiency while producing reproducible, consistent recoveries.

Keywords: Liquid Chromatography/Mass Spectroscopy, Pharmaceutical, Solid Phase Extraction, Water
Application Code: Pharmaceutical
Methodology Code: New Method
Analytical Challenges on Extractables Studies of Plastic Bioprocess Bags Using LC/MS and GC/MS

Single-use bioprocess bags are used for the preparation, storage and transport of biopharmaceutical solutions, intermediates and final bulk products. Most of the bioprocess bags are made of plastics and those plastics additives and degradants may easily leach out into the drug products. As part of safety risk assessment, it is very important to identify and quantify those extractables and leachables as they may pose safety risks to patients and/or change the efficacy of the medical products.

An extractable study was performed on plastic bioprocess bags by using different solvent systems, such as acidified water, alkaline water, PBS buffer, and organic/aqueous solvent mixtures to bracket and mimic pH values, ionic strength and hydrophobicity of common process fluids. In order to obtain a comprehensive extractable profile, multiple analytical techniques were used to identify and quantify organic extractables, including Headspace (HS)-GC-MS/FID analysis for volatile organic compounds, GC-MS/FID analysis for semi-volatile organic compounds, and LC-MS/UV analysis for non-volatile organic compounds.

During this study, there was one unexpected finding that the vapor of the volatile organic solvents in the plastic bags could migrate into the solvents in other bags. Modified experiments were then applied to investigate the root cause and effectively eliminated the problem. In addition, the diversified extractable profiles were compared and discussed for different extraction solvents. The results also demonstrate that High Resolution Accurate Mass (HRAM) and MS/MS data facilitate confident compound identification and unknown compound structure elucidation.

Keywords: GC-MS, Liquid Chromatography/Mass Spectroscopy, Pharmaceutical, Polymers & Plastics
Application Code: Pharmaceutical
Methodology Code: Liquid Chromatography/Mass Spectrometry
**Session Title:** Pharmaceutical - Chromatography and Mass Spectrometry  
**Abstract Title:** Coupling USP Method for Lidocaine and Prilocaine Cream with Mass Detection using Heart-Cutting Multi-Dimensional Chromatography  
**Primary Author:** Margaret Maziarz  
Waters Corporation  
**Co-Author(s):** Claude Mallet, Mark Wrona, Paul Rainville  

**Abstract Text:**

U.S. Pharmacopeia (USP) compendial methods are routinely adopted by pharmaceutical companies during the development and quality control of drug substances and finished drug products. Often, these methods use mobile phases with non-volatile buffers, which are not suitable for mass spectrometry (MS). In some cases, mass spectra data can be useful to accurately identify new or unknown components that may develop during the formulation process or routine testing.

Modifying USP methods to make them compatible with mass spectrometry is not allowable. In this case, a heart-cutting multi-dimensional chromatography with at-column dilution enables use of mass spectrometry directly with the unmodified USP methods. With this system, peak of interest is heart-cut from the USP method run on the first dimension (1D) and transferred into a holding loop. The heart-cut volume is then refocused onto a trap column (2D) using a dedicated pump configured for at-column dilution. After completion of the refocusing step, the peak of interest is desalted and ready for transfer to the third dimension (3D) using MS friendly mobile phase and analyzed by mass detection.

In this work, we demonstrate the capability of multi-dimensional chromatography for the use of mass spectrometry with an unmodified USP monograph for lidocaine and prilocaine cream. An impurity peak observed in the assay preparation solution is heart-cut, trapped, and analyzed in 3D LC to confirm identity by mass detection. In addition, the development of conditions for maximum peak trapping (trapping column chemistries and additives) will be discussed.

**Keywords:** Chromatography, Liquid Chromatography, Mass Spectrometry, Pharmaceutical  
**Application Code:** Pharmaceutical  
**Methodology Code:** Liquid Chromatography/Mass Spectrometry
Antibody-drug conjugates (ADC) are an increasingly important class of therapeutic agents in the treatment of cancer. ADCs are composed of a monoclonal antibody (mAB) and a cytotoxic agent (or “Payload”) that are combined together with a short synthetic linker. Due to the extreme toxicity of the Payload, the synthesis of the payload-linker complex is accomplished by manufacturing smaller pieces that are each individually less toxic. These pieces are then assembled in an order to generate the complex without forming the payload itself. During the manufacture of a specific payload-linker complex, a mass balance issue was encountered caused by the unexpected appearance of t-butylamine and hexafluorophosphate. This presentation discusses the variety of analytical techniques (GC-MS, Charged Aerosol Detector, Fluorine NMR) used to identify the contaminants in the material. Finally, this presentation will discuss the new control strategy required, including analytical performance of new methods used for the detection and quantitation of these two impurities.
Biopharmaceutical companies often need to transfer analytical liquid chromatography (LC) methods within their own organization and/or to external contract organizations. In many cases LC systems from multiple vendors must be utilized. It is important that system to system method transferability is demonstrated to minimize lost productivity. Thus, to ensure a successful method transfer, an understanding of the impact of instrument characteristics and specific method conditions on the separation is essential.

In this study, we will evaluate method transfer onto a novel biocompatible UHPLC system for analysis of proteins and digested monoclonal antibody (mAB) in size-exclusion (SEC) and reversed-phase (RP) chromatographic separations, respectively. Chromatographic system characteristics, such as extra-column volume, dwell volume and column heating, will be evaluated for the impact on both modes of separations. Method transfer will be conducted across systems from different vendors. For SEC separations, the impact of extra-column volume will be explored, including the effect of column heaters, tubing connections and detectors. For gradient separation mode, features of a biocompatible LC system will be employed to emulate other LC systems with different dwell volumes. In these examples, method transfer will be in accordance with regulatory guidelines for allowable adjustments to compendial methods.

**Keywords:** Biopharmaceutical, Liquid Chromatography, Method Development, Protein

**Application Code:** General Interest

**Methodology Code:** Liquid Chromatography
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<th>Session Title</th>
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<td>Full Three-dimensional Orientation and Position Determination of Single Anisotropic Nanoparticle with Dark Field Microscopy</td>
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| Primary Author | Xiaodong Cheng  
Georgia State University |
| Date | Wednesday, February 28, 2018 - Aft |
| Time |  
| Room | Exposition Floor, Aisles 2000-2700 |

**Abstract Text**

Resolving the orientation and position of single nanoparticle plays a significant role to understand the dynamics of the biological system. However, current full three-dimensional orientation methods mainly rely on determination complex imaging patterns, which is time-consuming and easily disturbed by the biological condition. Herein, we settle those problems by using dual channel dark field microscopy. By taking advantage of an auto feedback z adjustment system, we could obtain the precious z position by keeping the gold nanorods on the perfect focal plane. By inserting a half plane wedge prism into the optical path, the azimuth angles (from 0 to 360) could be resolved. Therefore, we successfully monitor the dynamics of transferrin-modified gold nanorods on the cell membrane. This new technique could be used to obtain valuable understandings on NP transport and endocytosis on the cell membrane.

**Keywords:** Biomedical, Imaging, Nanotechnology

**Application Code:** Biomedical

**Methodology Code:** Microscopy
Second harmonic generation (SHG) microscopy coupled with image analysis algorithms was found to enable the detection of crystals and quantification of crystallization kinetics in biomimetic media, including the highly turbid Ensure Plus [registered] system. The delivery of poorly water-soluble drugs using amorphous solid dispersions (ASDs) has been widely acknowledged as a promising strategy for enhancing oral bioavailability. Upon dissolution, ASDs have accelerated dissolution rates and yielded supersaturated solutions leading to higher apparent solubilities. Understanding the complex phase behavior of ASDs occurring during dissolution is crucial for developing an effective formulation. Since the absorption process of a lipophilic, high permeability drug is determined primarily by the intraluminal dissolution process, there is a need for evaluation in biorelevant dissolution media that simulate both fasting and fed gastrointestinal states. In this study, using ezetimibe as a model drug, three different ASDs were prepared using polyacrylic acid (PAA), polyvinyl pyrrolidone (PVP), and hydroxypropyl methylcellulose acetyl succinate (HPMC-AS). Dissolution of ASDs was carried out in sodium phosphate buffer, fed-state simulated intestinal fluid (FeSSIF), and Ensure Plus [registered] to evaluate the impact of different dissolution media on release profile, supersaturation and phase behavior. The supersaturation level and crystallization kinetics varied among the dispersions, and were found to be highly dependent on the medium employed. The presence of solubilizing additives in biorelevant media greatly affected the generation and stabilization of supersaturated solutions. Therefore, it is important to evaluate dissolution performance of ASDs in complex biorelevant media environments to better design supersaturating formulations for oral delivery.

The authors thankfully acknowledge funding for the work presented from the NIPTE, and the NSF-GOALI award.
Molecules confined in nanoreactors, either synthetically generated or those native to biological structures, can bring significant changes to their physical and chemical properties. In catalysis, nanoconfinement could dramatically change molecular transport and chemical conversion in porous materials, such as zeolites, mesoporous silica, carbon nanotubes, and metal-organic frameworks. Theoretical studies using simplified model systems, along with ensemble experimental measurements, have led to a limited understanding of the confinement factors, including the size, shape, and surface chemical properties of the pores on reaction kinetics. A significant breakthrough will be the development of single-molecule approaches to acquire direct experimental evidence and achieve quantitative understandings of the nanoconfinement effects at the single-molecule and single-nanopore level. In the past decade, molecular diffusion in nanopores have been visualized at the single-molecule level, and single turnover events on individual nanocatalysts have been mapped with nanometer precision by super-resolution microscopy imaging. However, molecular transport and reaction kinetics in nanopores have never been measured together experimentally due to seemingly insurmountable technical challenges of tracking single molecules dynamically in complex nanoporous structures under reaction conditions. In the present work, we designed a well-defined platform with catalytic centers confined in the end of nanopores with controlled lengths to study the in situ dynamic behavior of catalytic processes under nanoconfinement at the single-molecule and single-particle level. Variable single molecular mass transport behavior reveals the heterogeneity of the confined environment in the nanopores. The combination of the unique model catalyst and the single-molecule super-localization imaging technique paves a new way to understand the nanoconfinement effects in catalysis.
Localization Accuracy of Gold Nanoparticles in Single Particle Orientation and Rotational Tracking

Advances in Applications of Microscopy

The Single Particle Orientation and Rotational Tracking (SPORT) technique, which utilizes anisotropic plasmonic gold nanorods and differential interference contrast (DIC) microscopy, has shown potential as an effective alternative to fluorescence-based techniques to decipher rotational motions on the cellular and molecular levels. However, localizing gold nanorods from their DIC images with high accuracy and precision is more challenging than the procedures applied in fluorescence or scattering microscopy techniques due to the asymmetric DIC point spread function with bright and dark parts superimposed over a grey background. In this paper, localization accuracy and inherited uncertainties from unique DIC image patterns are elucidated with the assistance of computer simulation. These discussions provide guidance for researchers to properly evaluate their data and avoid making claims beyond the technical limits. The understanding of the intrinsic localization errors and the principle of DIC microscopy leads us to propose a new localization strategy that utilizes the experimentally-measured shear distance of the DIC microscope to improve the localization accuracy.

Keywords: Analysis, Imaging, Instrumentation, Microscopy
Application Code: Nanotechnology
Methodology Code: Microscopy
Molecular surfactants have been shown to control the shape of complex emulsion droplets, specifically oil-in-oil-in-water (o/o/w) double emulsions, when the concentration of the surfactant is varied. The use of particles, known as Pickering emulsions, to replace molecular surfactants has certain benefits since Pickering emulsions are often more stable and resist coalescence. In addition, Pickering emulsions have applications in many fields such as cosmetics, food, biomedicine, and several others. The purpose of this research was to test if Pickering emulsions could possess the same tunability and reconfigurability of morphologies that was seen in complex emulsions stabilized with molecular surfactants. The partial tunability of Pickering emulsions from a double morphology to a Janus morphology and back has been successfully achieved through the change in concentration of two different particle surfactants.

**Keywords:** Fluorescence, Lab-on-a-Chip/Microfluidics, Material Science, Modified Silica  
**Application Code:** Other  
**Methodology Code:** Microscopy
Application of Gas Chromatography/Mass Spectrometry

Smart Tune Wizard and Easy, Vent-Free Column Replacement - Two Innovations in Low-Resolution GC/MS Technology

Low resolution gas chromatography-mass spectrometry (GC/MS) is one of the most popular analytical techniques for analyzing compounds of interest in a variety of matrices. GC/MS is traditionally regarded as a complex analytical instrument requiring high level of user expertise to run. The two major pain points for the end user are tuning the MS and venting the system in order to change the GC column.

In this presentation we highlight two recent innovations that improve the usability of the GC/MS system with respect to the above mentioned points. Tuning of the mass spectrometer is a critical aspect of running the instrument that ensures the system is in the right working state and is ready to run samples. Mass calibration and resolution tuning are two of the most important steps that are performed to ensure that the system is in good working state. Most modern instruments have an AutoTune function that is fairly easy to use, but unnecessary tuning of the system is not recommended since it could result in shortening the lifetime of the detector. We present here the concept of the Smart Tune wizard which, with minimal clicks, checks the current tune state of the system and tunes it only when necessary. In case of tune/diagnostic failures, it gives specific recommendations to bring the system back into its recommended usable state. With the help of this wizard, a novice user would be able to start running their samples with minimal training.

The second innovation that we describe here is the vent-free column replacement. Mass spectrometers are maintained under high vacuum, thus changing the GC column requires the user to shut down the system, vent it, and bring the system back to steady state equilibrium, which often requires a day of down time. Current vent-free systems are prone to leaks and require high level of user training, however we present here an innovative vent-free column replacement technology that makes use of the vacuum probe interlock and is easy to use.

Keywords: Gas Chromatography/Mass Spectrometry, GC Columns, GC-MS, Instrumentation
Application Code: General Interest
Methodology Code: Gas Chromatography/Mass Spectrometry
Sample identification by GC-MS is typically performed with library search via the availability of fragment ions, automatically providing compound names and identification probabilities. However many compounds are incompatible with GC-MS analyses (do not elute) and about 30% of the compounds exhibit weak or no molecular ion hence their library identification can-not be trusted plus many compounds are not included in the library. Significantly improved methods of sample identification were explored using the Aviv Analytical 5977-SMB GC-MS with Cold EI, an instrument based on the use of supersonic molecular beams (SMB) for interfacing the GC and MS with electron ionization (EI) of vibrationally cold sample compounds in SMB in a fly-through ion source (hence the name Cold EI).

The following sample identification aspects were improved:
A) The use of short columns with high column flow rates enables significant extension of the range of compounds amenable for GC-MS analysis, while the fly-through ion source ensures their ionization without ion source degradation, thereby extending the range of compounds amenable for GC-MS identification.
B) Enhanced molecular ions improve the library identification probabilities and confidence in identification.
C) The combined availability of enhanced molecular ions and Tal-Aviv Molecule Identifier (TAMI) software based on isotope abundance analysis and improved quadrupole mass accuracy serves to effectively provide elemental formula.

Thus, GC-MS with Cold EI and TAMI software is superior to GC-HR-TOF in sample identification since greater range of compounds can be analyzed and exhibit trustworthy molecular ion with it, while the TAMI software provides elemental formula.
Over 1000 compounds from a wide range of chemical classes are known to contribute to the aroma of whisky, and these include alcohols, phenolics, fatty acids, esters, lactones, aldehydes and nitrogen-containing compounds. It is important to be able to confidently identify these volatiles, for quality control and authentication purposes, as well as in the engineering of new aromas.

Comprehensive two-dimensional gas chromatography coupled with time-of-flight mass spectrometry (GCxGC–TOF MS) is ideal for the analysis of such complex samples, because the enhanced separation capacity allows analysts to screen the entire composition in a single analysis, with confident identification of compounds that would ordinarily co-elute.

This study investigates the application of flow-modulated GCxGC–TOF MS using a reverse-fill/flush device, which allows separation of volatiles ranging from C1 to C40 (and above), the flexibility to change the loop volume in method optimisation, splitting for simultaneous detection and efficient modulation of whisky volatiles that have boiling points similar to, or lower than, pentane.

Keywords: Food Science, Gas Chromatography/Mass Spectrometry, Separation Sciences, Volatile Organic Compounds

Application Code: Food Science
Methodology Code: Gas Chromatography/Mass Spectrometry
The ingestion of Epichlorohydrin (ECH) is toxic in drinking water and has a possibility of causing cancer if a large amount is consumed. According to the World Health Organization the maximum concentration allowed in drinking water is 0.4 µg/L. In 1974, Congress passed the Safe Drinking Water Act which requires the Environmental Protection Agency (EPA) to determine the level of all contaminants in drinking water to where no health effects are likely to occur. The maximum concentration permissible in drinking water regulated by the EPA is 0.1µg/L. This application will compare how purge and trap sampling and Solid Phase Micro-Extraction (SPME) detect ECH in water samples.
Environmental forensics is concerned with the source, fate and transport of contaminants in the environment. Analytical instrumentation is constantly evolving, thus new protocols to trace the origin of contamination must also be developed to utilise these technological advances. Traditionally, the detection of trace-level compounds in such samples has been performed by quadrupole MS in either full scan or selected ion monitoring (SIM) mode. However, qMS is a mass filter, with a high percentage of ions being wasted, thus limiting sensitivity. Moreover, in SIM mode, only target compounds can be monitored, meaning that full characterisation of the sample is not possible in a single run, and retrospective searching of data is limited.

The use of time-of-flight mass spectrometry (TOF MS) can overcome this issue by providing highly sensitive detection whilst acquiring full-range mass spectra, to allow both target and unknown identification in a single, rapid analysis. Nevertheless, acceptance of TOF technology has been restricted due to large instrument size and the production of spectra exhibiting mass discrimination.

This poster describes an innovative, robust TOF MS system that eliminates these problems. The generation of classical EI spectra allows traditional library searching for confident identification of trace-level compounds.

Here, the complementary use of GC–TOF MS with a flexible thermal desorption (TD) platform is detailed for the analysis of a range of contaminants across a variety of environmental matrices.
During volatile analysis, samples are purged with an inert gas in order for the Volatile Organic Compounds (VOCs) to be swept out of the sample and onto an analytical trap. Purge flows and times are recommended by the United States Environmental Protection Agency (USEPA). For years the USEPA recommended purge flow and time was 40ml/min for 11 minutes, however in USEPA Method 524.3 and 4 there is approval to vary purge flows and times. Many laboratories would like this consent for their ground water and soil samples using USEPA Method 8260. Since purge flows and times can have an impact on the analytes of interest, this application will investigate and optimize these parameters.
The need for accurate monitoring of SVOCs in surface and waste water samples using methods such as US EPA Method 8270 continues to grow in importance as the comprehension of their adverse effects on human health evolves. Improvements in analytical technology for accurate determination down to sub-PPB levels is critical to obtain the most comprehensive monitoring possible. Current techniques for extraction of base/neutral/acid SVOCs include solvent extraction and separatory funnel extraction, however these methods require many steps and environmentally unfriendly solvents. Additionally, there are many analytical challenges due to matrix interferences, contamination, and the broad range of chemical properties. A new solvent-free automated technique for extracting SVOCs in water using Vacuum Assisted Sorbent Extraction (VASE) paired with GCMS is presented. VASE allows reliable extraction with minimal matrix effects due to the high phase ratio and surface area of the adsorbent. VASE is a powerful extraction technique which places a sample vial under vacuum in the presence of a 70mg adsorbent cartridge (Sorbent Pen) to effect near exhaustive extractions of GC compatible compounds. After the vacuum source is removed, the vial remains under vacuum causing transfer to the adsorbent faster than at atmospheric or higher pressures. Repeated heating and cooling of the sample creates a boiling action in a closed system which effectively transfers even the heaviest SVOCs and recovery surrogate standards into the adsorbent. Sample injection is performed with a thermal desorption unit fitted into a GC injection port. Extracted compounds remain near the front of the adsorbent ensuring their quantitative recovery. Calibration curves for SVOCs such as phthalates, pesticides, PCBs, and PAHs and results from surface and waste water samples are presented. Data reveals both the reproducibility and lack of carryover achieved by this new technique resulting in accurate analysis of SVOCs.

Keywords: Environmental/Waste/Sludge, Environmental/Water, Extraction, Thermal Desorption
Application Code: Environmental
Methodology Code: Gas Chromatography/Mass Spectrometry
The Combination of 3 New Sampling Techniques for Determination of Uptake Rates and Monitoring of Semi-Volatile Organic Compounds (SVOCs) in Indoor Air

One of the authors of this abstract to be submitted for a poster presentation is out of the country and communication is extremely limited. We will submit the abstract for this poster presentation by September 1st, 2017 after his return. We understand we may be placed on a waiting list due to a late submission, but we are writing to you before submission closing in hope that we can still hold a place with a late submission due to unforeseen circumstances. I have also sent an email to Becky Hackley. Thank you for your consideration.

Keywords: Environmental Analysis, Environmental/Air, Sampling, Semi-Volatiles
Application Code: Environmental
Methodology Code: Gas Chromatography/Mass Spectrometry
Abstract

US EPA Method 325 has been developed to enable refineries to comply with the updated US federal regulation CFR 40, and requires monitoring of VOCs around the boundary of refineries. This involves two-week passive (diffusive) sampling onto sorbent tubes, followed by analysis by thermal desorption (TD) in conjunction with gas chromatography (GC), with analysis by mass spectrometry (MS) or other detection technique. While benzene is the primary target compound, the sampling and analysis methodology can also be used to determine other VOCs, including butadiene, toluene, ethylbenzene, xylenes and other hazardous air pollutants (HAPs).

This poster describes a stepwise approach to complying with US EPA Method 325 for monitoring volatile organic compounds (VOCs) at refinery perimeters. A range of equipment from Markes International is outlined that allows fully method-compliant deployment of tube-based passive samplers, sample analysis and tube cleaning. All these stages are underpinned by a radio-frequency identification tagging system to ensure a robust chain of custody from field to lab.

Keywords: Air, Environmental/Air, GC-MS, Thermal Desorption
Application Code: Environmental
Methodology Code: Gas Chromatography/Mass Spectrometry
Menthol is a naturally-occurring substance found in various mint plants. Menthol’s flavoring, cooling, and analgesic properties have provided its use in a variety of consumer products including candies, medicines, and other personal care products. Its addition to cigarettes, however, remains a significant health concern due to its cooling sensory effects, thus making smoke inhalation smoother and easier for smokers. Early marketing strategies promoted the smoking of mentholated cigarettes as a healthier and safer alternative to non-mentholated cigarettes and targeted populations with specific demographics (i.e., beginning smokers, smokers with health concerns, and African Americans smokers). Various analytical methods measuring menthol levels in unburned cigarette tobacco have been reported, but little has been reported for measuring menthol in mainstream cigarette smoke. A sensitive and selective method for measuring menthol deliveries of machine-generated mainstream mentholated cigarette smoke is presented here. Mentholated cigarettes were machine-smoked under ISO (35 mL puff volume, 2 sec puff duration, and 60 sec puff intervals) and Canadian Intense (55 mL puff volume, 2 sec puff duration, and 30 sec puff intervals) smoking regimes, and the resulting trapped smoke particulate matter was extracted and analyzed via isotope dilution gas chromatography-mass spectrometry (GC-MS). Method accuracy and precision ranged 82.2%–94.1% and 4.9–7.8%, respectively, between low, mid, and high menthol-spiked ISO and Canadian Intense 3R4F reference cigarette smoke samples. Menthol deliveries from select mentholated cigarette brands were found to decrease by as much as 11%-28% after just 6 hours after pack opening, and 44%–58% after 72 hours.

Keywords: Analysis, Consumer Products, GC-MS, Validation
Application Code: Consumer Products
Methodology Code: Gas Chromatography/Mass Spectrometry
Compound specific stable isotope analysis recently was one of the main applied tools in metabolomics studies. Amino acid (AA) specific isotope analysis of biological extracts may help to understand development or find specific markers for diseases or other stressors. One of these stressors, which is still widely researched, is oxidative stress. This study deals with development and application of methods needed to perform and master isotopic analysis with gas chromatography followed by mass spectroscopy or isotope ratio mass spectroscopy. Herein described experiments deals with development and application of AA extraction, purification, derivatization and gas chromatography methods.

Standardized oxidative stress method was developed and used to collect affected yeast Saccharomyces cerevisiae cells. Stable isotope analysis was performed on AA extracts acquired from different yeast strains grown in elevated oxidative stress conditions. Evaluated different and strain-unique AA isotope ratio shifts in cells grown on stress. Results show that oxidative stress does greater impact on isotope ratio in those yeast strains which does not possess catalase type enzymes. Isotopic ratios of 15N/14N and 13C/12C are prone to change more in mentioned strains compared to those which did have catalases. AA specific isotope analysis were also performed on AA extracted from different AA domains. It was found out that AA undergo larger isotopic shift in those domains, which are more dynamic: free AA and AA of soluble proteome. Though AA which belong to insoluble proteome are more resilient to isotopic changes.

Keywords: Biological Samples, Gas Chromatography/Mass Spectrometry, Isotope Ratio MS
Application Code: Genomics, Proteomics and Other 'Omics
Methodology Code: Gas Chromatography/Mass Spectrometry
Development of Portable Instrumentation

Development of Electrochemical Immunochromatography Platform as Point-of-Care Testing device

Point-of-care testing (POCT) is an easy, fast, and low-cost diagnostic method, and it is expected to be used at the bedside, near the patient, in the agriculture fields, and environmental measurement. Especially in agricultural field, monitoring of biomarkers such as estrogens and luteinizing hormone (LH) are important for effective production of livestock leading to profitable management. Therefore, we developed easy to use electrochemical immunochromatography platform as POCT device. We succeeded to add quantitative capability to immunochromatography by applying electrochemical technic. The platform is easily connected to USB type potensiostat and tablet PC by inserting the USB connector to the platform.

In this study, we measured progesterone that is very important to monitor estrus cycle of livestock. At first, progesterone and biotin labeled progesterone were injected to the platform. Unlabeled progesterone competed with labeled progesterone to bind to antibody. Unbound progesterone were washed away by next washing step. After that, streptavidin labeled alkaline phosphatase (ALP) was injected. The streptavidin bound to biotin and formed conjugate. Unbound ALP was also washed away by second washing step. Finally, $[i]p[/i]$-aminophenyl phosphate ($[i]p[/i]$APP) was injected and reacted with ALP and produced $[i]p[/i]$-amino phenol ($[i]p[/i]$AP). The amount of pAP was measured by electrochemical detector. Chronoamperometry with applied potential was 0.2 V vs Ag/AgCl was started when the $[i]p[/i]$APP was injected to the membrane. Chronoamperometric signal showed peak signal when the produced $[i]p[/i]$AP arrived to electrode. The height of peak oxidation current was correlated with concentration of progesterone. This result suggests that our device can measure the concentration of progesterone.

Abstract Text

In conclusion, we developed a novel electrochemical immunochromatography platform for point-of-care testing. The platform is portable and easy to use, and we were able to add quantitative capability to immunochromatography by applying electrochemical technic. This device is expected to be useful for monitoring of biomarkers in various fields, including agriculture and environmental measurement.

Keywords: Electrochemistry, Immunoassay, Portable Instruments, Sensors
Application Code: Agriculture
Methodology Code: Portable Instruments
Open-type absorption direct measurements on a microwell-plate were proposed and demonstrated by using silicone optical technology (SOT). No optical covers to blocking outside background light except small top cover was needed. The spatial filter using silicone material is attached on the bottom of the microwell, and collect only the light from a white LED and via the sample liquid in the microwell. A simple RGB sensor was used for optical detection like our previous work about PiCOEXPLORER (PAS-110, Ushio Inc.). As shown in the figure, the optical filter was carbon dispersed polydimethylsiloxane (KE-COLOR-BL 10wt% in SIM-360, Shinetsu Chemical) module contains transparent PDMS  (1mm diameter, 6mm length, SIM-360, Shinetsu Chemical) as an optical channel. This filter can trap light with tilted incident angle, so stray light coming from multiple internal scattering of the polystyrene of the microwell-plate can be expected to reduce significantly. In result, an only small light block over the wells as shown in Figure can suppress the background radiation without side light blocking walls. It means that a very compact optical detection attaching device will be possible such as just-sized on the bottom of the well. In the preliminary experiment, the circumstance background light without LED lighting was counted as 10 in 16bit A/D converter from the RGB-sensor and in comparison with the bright and the dark room. On the other hand, LED lighting with maximum power in the software of PAS-110 obtained about 5.4×10^4 counts can be trapped with red channel measurement using the RGB sensor with the spatial filter, and the background radiation was corresponding to 0.02% of the signal level. On the other hand, the sample amount of 100~300 μL was varied for evaluation, and the signal level was not affected by the amount. Thus, 10^-4 absorption measurement can be expected with this scheme in future.
Development of Portable Instrumentation

Combination of Ion Mobility Spectroscopy with Optical Detection Methods to Improve the Detection Capabilities and the False Alarm Rate

Ion mobility spectrometers (IMS) are systems which can detect many different toxic industrial chemicals in very low concentrations. Their ambient pressure operation makes them suitable for small, portable hand held systems. A disadvantage is their low resolution, compared e.g. to mass spectroscopy that leads to possible false alarms due to compounds with similar drift times, i.e. mobility. When combining IMS with other detection methods, e.g. optical detection techniques the false alarm rate can be significantly reduced. Another advantage is, that the range of detectable compounds can also be enhanced, because IMS alone cannot detect all compounds of interest.

In this talk we will present first research results on the selection of optical detection methods for the combination with IMS. The optical methods have to have comparable detection limits and response times too IMS to maximize the benefits of both orthogonal methods. We evaluated a photoionization based detection system and a flame photometric based detection system regarding their performance when detecting selected toxic industrial chemicals.

Subsequent we linked the flame photometric based detection to atom emission spectroscopy (AES) to enhance the potential by evaluating the optical emission spectra. Accordingly we will present the results of combined evaluation of IMS drift time spectra and AES spectra. We will introduce a novel approach of combining both orthogonal methods in one instrument.

Keywords: Atomic Emission Spectroscopy, Integrated Sensor Systems, Spectroscopy, Trace Analysis
Application Code: Homeland Security/Forensics
Methodology Code: Integrated Sensor Systems
The Gas Chromatographic (GC) separation of isotopic compounds is traditionally done on polysiloxane based stationary phases. The traditional polysiloxane based GC stationary phases show inverse isotope effect, meaning deuterated isomer elutes earlier than the nondeuterated isomer. Conversely, Ionic liquid (IL) columns were showed to have marginal inverse to normal isotopic effect. Also, the separation is dependent on the polarities of IL stationary phases. A series different IL columns with different selectivities and polarities were developed and analyzed in the separation of different isotopic compounds. The effects of IL polarities and structural variation on the selectivities and resolution of isotopic compounds were studied. The separation of deuterated and nondeuterated compounds is mainly dependent on the van der Waals dispersion forces. So, a class of ILs with partially fluorinated linkage chains and higher van der Waals interaction were also synthesized. The GC columns coated with partially fluorinated ILs then were evaluated in the separation of different deuterated compounds.
The analysis of ethylene glycol in water is a common test performed by food safety, chemical and environmental laboratories. Many environmental samples originate from water runoff at airports, where ethylene glycol is used as a de-icing agent for airplanes during winter months. Because ethylene glycol is highly soluble in water, it is not easily concentrated by purge and trap. Therefore, the most frequently used sample introduction technique is direct aqueous injection. The direct aqueous injection of ethylene glycol is challenging because it can be difficult to attain reproducibility and good peak shape. The large expansion volume of water can cause backflash, carryover can cause inconsistent results, and excess water can extinguish the FID flame. These problems can prevent achieving the detection limit for ethylene glycol, which may vary in the 1-10 ppm range. This study compares lifetimes of various PEG (polyethyleneglycol) stationary phases under optimized conditions.
Gas Chromatography

Let's Analyze Live Crude from C1 to C100

This research provides a gas chromatography (GC) simulated distillation (SimDis) technique for the boiling point distribution of a live crude oil from C1 to C100. The referee technique for this boiling point range on a stabilized crude is ASTM D7169, and recently ASTM D7900, which provides enhanced accuracy in the boiling point range by resolving the quenching effect of carbon disulfide the solvent used to dilute the sample.

A “live” crude is when the sample is preserved so that the light ends or more volatile components are not lost in collection and in sample transfer. This is critically important for light crudes and/or Bakken crudes where the sample has a significant amount of lower boiling components, and this preservation provides a more accurate result of boiling point distribution. Samples are taken in the field in floating piston cylinder to prevent loses of the volatile components.

ASTM D8003 explains how the samples are injected into the GC to prevent loses of the volatile components; however, ASTM D8003 only covers C24 minus. Using this injection technique, and micro fluidic dean switching technology, this presentation discusses the success of attaining the full boiling point distribution range on a “live” crude.

Keywords: Fuels\Energy\Petrochemical, Gas Chromatography, Sample Introduction
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Gas Chromatography
Ionic liquids (ILs) have attracted significant attention in different branches of science and industry. Thanks to their unique physical and chemical properties, these leading-edge materials have successfully utilized as GC stationary phase. Commercialized IL-based GC columns demonstrated excellent selectivity for separation of different class of volatile and semi-volatile compounds. Despite a considerable number of published reports utilizing ionic liquid stationary phases, there is still lack of sufficient knowledge on structural components of these materials playing principal role in separation processes. Therefore, it seems necessary to carry out an extensive investigation on influence of different structural variations on selectivity and polarity of ionic liquid platforms. We studied the effect of 3 factors on separation properties of dicationic ionic liquid stationary phases. First, we showed how altering the cationic head groups can affect the selectivity of IL-based stationary phases. Next, the influence of different types of spacer chains was studied. Different types of fatty acid methyl ester (FAME) mixtures were analyzed to evaluate the polarity, and selectivity of ionic liquid columns. Lastly, the effect of different anions on retention behavior of fatty acids and aromatic compounds was investigated. Overall, 30 novel dicationic ionic liquids were synthesized and examined as GC stationary phase. Moreover, McReynolds constants were determined to compare the polarity of these columns.

We appreciatively acknowledge the Robert A. Welch Foundation (Y0026) for the support of this work.

Keywords: Capillary GC, Food Science, GC Columns
Application Code: Food Science
Methodology Code: Gas Chromatography
Organotin compounds have a great many applications which include stabilizers, catalysts, biocides, and pesticides. One of the more significant uses was that of biocides employed as anti-fouling paint on sea-going vessels which has contributed to coastline contamination throughout the world. Although their use as a biocide in anti-fouling paint was completely banned in 2008, residual contamination is still present in water and sediment. Organotins are also significant in the plastics industry because they are used in place of heavy metals such as lead and cadmium. They are an important heat stabilizer additive in the manufacture of PVC. Organotins have been found to bioaccumulate and cause a number of health related problems including being a possible endocrine disrupting compound. Many laboratories are faced with requests for organotin analysis as public awareness of the toxicity of these compounds grows. Presently their use is strongly regulated because of the high degree of toxicity. Unfortunately there is no EPA accepted method for this analysis and many labs pass up work or subcontract the analysis to one of the few labs that analyze for organotin because they are under the impression that it would be a great capital expense and take a great deal of expertise to successfully run the analysis.

The purpose of this poster is to present a sensitive, selective method which may easily be incorporated into laboratories day to day analyses. The PFPD offers ppb sensitivity along with great selectivity for tin versus hydrocarbons. The derivatization procedure using sodium tetraethylborate alkylation for the standards, optimum GC-PFPD conditions, and calibration data will be presented. Sample data for blood plasma, urine, wastewater, and dewatered sludge will also be presented.

Keywords: Derivatization, Environmental Analysis, Environmental/Biological Samples, Gas Chromatography
Application Code: Environmental
Methodology Code: Gas Chromatography
This study reports a method of searching multiple-source for VOC contaminations in a school surrounded by industrial area using dual real-time µGCs and an anemoscope. The µGCs consist of a multi-stage preconcentrator, a 15 m column and a commercially available photon ionization detector (PID) unit (Alphasense, UK) were assembled in our lab which is capable of analyzing sub-ppb VOCs. Two µGC sampling sites are distanced by 80 meter were placed on the roof of the school building in a diagonal position. The anemoscope was used to record the wind direction and velocity simultaneously with µGC analyses. A canister/GC-MS sample analysis was performed to provide the identifications of VOC species and time-averaged concentration data as the reference for µGCs measurements. The µGCs continuously measured VOCs with each cycle within 15 minutes for 16 days. Seven VOC compounds including acetone, methyl acetate, butanone, n-hexane, benzene, toluene and xylene were found in concentration range of 0.1 to 550 ppb. Individual VOC concentration trend in the field showed log-normal distribution (n > 1500 data for each compound). The spatial probability of VOC emissions contour was calculated by triangulation of direction-dependent possibilities from two side-by-side µGC concentration data combining with wind direction. The hot spot on this contour can be expected to be the emission source. Compounds such acetone, methyl acetate, butanone, benzene and toluene, were found the probability direction of southwest, which match the surrounding chemical plants. By using two in-field µGCs and an anemoscope for continuous analysis, this method can rapidly accumulate large numbers of wind direction dependent low-ppb concentration data for various chemicals. With proper calculation and interpretation into spatial probabilities, this method is useful for assessing the impacts of multiple VOCs released from different part of industrial area to nearby residences.
Gas chromatography is the most popular chemical analysis tool in the world due to its reliability, resolving power, speed, and sensitivity. In this paper, we will investigate the use of large volume injection (LVI) with solvent elimination in order to dramatically improve the sensitivity on a standard gas chromatograph. Most GC applications are conducted in the split mode, whereby the analysis splits the sample in the inlet and throws away 90-99% of the sample to avoid overloading the column. While split is the preferred approach for most samples, sensitivity suffers dramatically.

The first step to improving sensitivity of a split injection is simply to lower the split ratio. If the current ratio is 100:1, changing it to 10:1 produces roughly 10 times the sensitivity (a little less due to increased peak broadening).

The next step is to consider splitless injection. For this approach to work, the analyst must carefully choose the sample solvent and the initial column temperature to take advantage of the solvent effect (keep early-eluting peaks from broadening). Splitless provides roughly another 10 times more sensitivity than a 10:1 split.

With a little luck, you may be able to inject 2 uL in the splitless mode, but increasing the injection volume further usually results in unacceptable peak shapes.

However, if the analyst could vent the solvent before the column while holding onto the compounds of interest, the injection volume is almost limitless. In this study, we investigate the limits and real-world applicability of large volume injections with an Agilent Multi Mode Inlet (MMI) installed onto a modern GC, including the effect of injection volume on peak shape, sensitivity, and thermal breakdown.
Formaldehyde is a known carcinogen commonly found in foods, treated wood, and a variety of other household products. It can also be found in soil near burial grounds, glue, plywood, fiberboard insulation, and car exhaust. OHSA established an exposure limit of 0.75 ppm on average over an 8-hour work day. Measuring formaldehyde by gas chromatography is difficult due to the lack of response in the flame ionization detector. Historically, formaldehyde determination required the addition of a mass spectrometer (MS) or photo ionization detector (PID). There are HPLC methods, but those require the use of post column derivatization to detect the formaldehyde.

In this study, we investigate the use of a PolyArc two stage reactor in line with a flame ionization detector to measure the presence of formaldehyde. The first stage of the reactor oxidizes the formaldehyde (along with all the other carbon-containing compounds) into carbon dioxide. The second stage employs a proprietary catalyst in a hydrogen-rich environment to convert the carbon dioxide to methane, which is then easily detected by the FID. We combine these enhanced detection capabilities with a speed-optimized static headspace GC method to determine low levels of formaldehyde and other compounds in a variety of consumer products. With advances in analytical instrumentation, formaldehyde testing will become more accessible to the industries that need it.

Keywords: Gas Chromatography, GC, GC Detectors, Industrial Hygiene
Application Code: Industrial Hygiene
Methodology Code: Gas Chromatography
The choice of an inlet liner is critical to GC analysts, since it impacts the quality of the chromatographic data. Inertness is one of the most important factors to consider, as active analytes can easily be degraded or adsorbed within a hot GC inlet. A new liner deactivation was developed with a high level of inertness towards sensitive compounds. This liner deactivation was tested with a variety of analytes, including various classes of pesticides, as well as acidic and basic probes. Other liner deactivations were also analyzed to compare performance for active compounds. Liners used in this study were single taper with wool and all injections were performed in splitless mode. This provides one of the most rugged assessments of deactivation quality.
Introduction of analytes into a gas chromatograph typically occurs through a heated inlet, containing a glass liner. Inlet liners are deactivated to prevent unwanted interactions from occurring between the analytes and active sites commonly found in raw glass. These adverse reactions include chemical breakdown of reactive compounds as well as reversible and irreversible adsorption.

A new liner and wool deactivation has been developed with a high degree of inertness for many classes of compounds. This deactivation utilizes a vapor deposition process that results in a consistent coating with reproducible chromatographic results. The following work demonstrates the importance of using an inert inlet liner with repeatable performance from lot to lot. Examples of reactivity present in inlet liners will be presented and explored, emphasizing the benefits of the new liner deactivation.

**Keywords:** Gas Chromatography, Sample Introduction, Trace Analysis

**Application Code:** General Interest

**Methodology Code:** Gas Chromatography
According to Van Deemter, slower flow rates yield greater separation power, but slower analysis times. We can still get reasonable times if we adjust to a smaller number of plates. I will show good separating power in a fairly short timeframe, using pressure adjustment in concert with short column length. While I demonstrate using basic gas analysis, the principles could be expanded for other GC analyses. Support from Valco/VICI and University at Buffalo is deeply appreciated.
Gas chromatography (GC) is an analytical tool that is widely used in the analysis of mixtures of volatile or semi-volatile compounds. While GC can be used to separate and detect individual components of a complex mixture, there are some applications where partially separated peaks using ultrafast GC can be employed for discrimination and classification with the aid of pattern recognition techniques. For this purpose, herein, we report a new multi-channel micro gas chromatography separation column that enables discrimination and classification of a number of closely related test mixtures. The channels of the separation column utilize different topographies and room temperature ionic liquid (RTIL) stationary phases in order to increase the amount of information that can be obtained from ultrafast separations. By applying statistical models to the experimentally acquired data, we have shown prediction accuracies of more than eighty percent. We are currently working to increase the prediction accuracy by utilizing different topographies and RTIL stationary phases with different selectivities. This technology will eventually be used for ultra fast analysis of food adulteration.

Keywords: Food Identification, Gas Chromatography, GC Columns
Application Code: Food Identification
Methodology Code: Gas Chromatography
High throughput testing in catalysis and, especially in heterogeneous catalysis, is a well-established but still a fast-growing field for the chemical and petrochemical industry. Besides the parallelized reactor systems, a lot of attention was given to the development of fast, reliable, accurate, and detailed analysis of product streams from testing units. In many cases, online analytics is the bottleneck regarding throughput since analytical methods, i.e. online gas chromatography (GC), intrinsically have long durations in order to achieve sufficient data resolution. Thus, it is mandatory to improve and optimize existing GC hardware and methods regarding accuracy and throughput.

In this contribution, we present selected highlights of GC method developments in recent years with unprecedented speed and accuracy in complex and challenging applications. In particular, petrochemical processes exhibit complex product streams consisting of up to 300 components that have to be identified and quantified in the shortest time possible in order to maximize catalyst testing throughput. An outstanding example of fast online analytics including short sampling intervals is the parallelized testing of naphtha reforming catalysts. A full range naphtha analysis via online GC enabled an automatized calculation of research octane numbers (RON) from measured chromatograms. The results were used for an automatized temperature adjustment of each catalyst reactor position until an identical RON was achieved for all reactors. A requirement for this new development was the extremely fast quantification of all components in a sophisticated multi-column / multi-detector GC set-up.

Keywords: Gas Chromatography/Mass Spectrometry, High Throughput Chemical Analysis
Application Code: High-Throughput Chemical Analysis
Methodology Code: Gas Chromatography/Mass Spectrometry
Gas Chromatography

Expanding the Horizons of Headspace GC with Fast Simultaneous Water and Solvent Quantitation in Pharmaceuticals

Water quantitation by coulometric Karl Fischer titration is an industry standard due to its high degree of specificity and accuracy. However, water content is often underestimated for ketone and aldehyde samples, which react with the reagents used in the technique. The analysis loses reliability for samples with >2% water, and the oven temperature required to release water varies with each sample and can fail to release water of crystallization. These sources of error necessitate the reassessment of data, are poorly traceable, and significantly impede method transfer.

Headspace capillary GC is a robust technique optimal for residual solvent determination in pharmaceuticals, but is not typically used to quantitate water since the common flame ionization detector (FID) yields no response. While thermal conductivity detectors (TCD) are sensitive to water, interference from absorbed water in diluents for headspace GC makes accurate quantitation difficult. However, we have demonstrated accuracy matching that of Karl Fischer by developing techniques to control water content in the blank at consistent and low levels.

Since a wide variety of solvents are used in the pharmaceutical industry, generic methods for their detection should be able to resolve dozens of solvents down to well below their ICH limits. However, resolution of that many solvents with widely different volatility and polarity requires methods with long run time, and sample analysis typically takes several hours. By switching to hydrogen as a carrier gas, much faster methods can be developed that maintain equal or better resolution over a wider linear velocity range.

We have developed and validated a high-speed headspace GC method with hydrogen carrier gas for quantitating water, other non-carbonaceous compounds, and over 25 residual solvents within the same analysis. The method has been assessed for linearity, precision, and sensitivity of all compounds and is applicable to APIs and drug products.

Keywords: Gas Chromatography, Pharmaceutical, Solvent, Water
Application Code: Pharmaceutical
Methodology Code: Gas Chromatography
Integration of solid phase extraction with liquid chromatographic (LC) analysis may take two approaches: 1) Direct coupling, in which a sample loaded in a cleanup column is completely transferred to the analytical column using a switching valve; 2) Indirect coupling, in which samples are treated like in standalone SPE. A portion of the collected fraction is then injected into the HPLC. The direct approach can give high sensitivity and fast sample throughput since the entire cleaned up sample is used for final analysis and the procedure is simple. The problem with the direct approach is finding a suitable SPE column that is compatible with the analytical column and can be used for many samples, as it is not easy to change an SPE column that is fixed to a high pressure switching valve. On the other hand, the indirect approach can avoid this problem as the SPE column is decoupled from the HPLC column and only a small volume is used for HPLC analysis.

We here introduce a fully automated two-tier online SPE approach to overcome the disadvantages of the two approaches described above. In a 2-tier online SPE, a sample is first extracted using a 3-mL or 6-mL SPE column like in a standalone SPE. The collected fraction is then mixed with one or two solvents. The fraction is then loaded onto the second SPE column fixed on a switching valve and followed by more cleanup. All the trapped sample is then transferred to the LC column for final analysis. The first SPE treatment is used to remove most of the interference. The second SPE column is used for enrichment and further cleanup. Since the fraction entering the second SPE column has been cleaned by the first SPE column, the lifetime of the second column is considerably extended. Besides, using the solvent modulation and the enrichment of the second SPE column, a volume of 500 μL of sample solution of containing chloramphenicol and caffeine in methanol can be introduced into a C18 column without peak broadening.

Keywords: Automation, Environmental Analysis, Liquid Chromatography/Mass Spectroscopy, Solid Phase Extraction
Application Code: General Interest
Methodology Code: Liquid Chromatography/Mass Spectrometry
Canola is a major crop product in Canada used for the production of edible oil. During production, significant amount of waste is generated, termed the deodorizer distillate (DD). This low value waste is rich in biologically active metabolites, namely phytosterols and tocopherols. Therefore, efficient extraction of these metabolites represents an economical opportunity. Regardless of the extraction strategy, the amount and type of metabolites should be evaluated. We are developing liquid chromatography-tandem mass spectrometry (LC-MS-MS) method for the analysis of four phytosterols and four tocopherols.

Collision induced dissociation (CID)-MS/MS analysis was performed using quadrupole-linear ion trap and an orbitrap MS instruments. Complex spectra were obtained for phytosterols while the number of product ions was limited in the case of tocopherols. Phytosterols dissociation was initiated on the side chain followed by multiple ring cleavages. MS/MS analysis of tocopherols was centered on the aromatic hydrocarbon ring resulting in relatively small product ions. The fragmentation pathway and the genesis of each product ion were validated through exact mass measurements as well as second generation MS3 analysis.

HPLC-MS/MS method is being developed to determine the target metabolites within DD extracts. C18 column is used with isocratic mobile phase of acetonitrile/methanol (99:1 v/v with 0.01% acetic acid) with a run time of 6.5 minutes. Cholestan-3ol and RACT-OCOL were used as internal standards for phytosterols and tocopherols, respectively. Preliminary data showed that hexane-based extraction of DD produced similar amounts of phytosterols in comparison to “friendly” extraction strategies, using reagents suitable for human consumption. In addition, DD obtained from cold-pressed seeds was richer in phytosterols than hot-pressed ones. Finally, precursor ion scan, exact mass measurement and MS/MS are used for the identification of additional unknown peaks.

Keywords: Agricultural, Chromatography, Tandem Mass Spec
Application Code: Agriculture
Methodology Code: Liquid Chromatography/Mass Spectrometry
Mycotoxins are one of the major food and feed contaminants that have negative effects on human health, including immunosuppressive, carcinogenic, nephrotoxic, and hepatotoxic effects. To monitor population exposure to mycotoxins, it is important to develop sensitive LC-MS multi-mycotoxin assay allowing simultaneous detection and quantification of 17 toxicologically important mycotoxins and their metabolites in human plasma.

The optimization of LC-MS method used LTQ Orbitrap Velos and pentafluorophenyl reversed-phase LC. Mobile phase containing water/methanol with 0.1% acetic acid and water/methanol with 0.02% acetic acid were used in positive and negative electrospray ionization, respectively, for the separation of mycotoxins, including isomers. The use of 0.02% acetic acid in negative mode was also found to enhance ionization from 1.7 to 26 times.

After comparison of analyte recovery, selectivity, and matrix effects with different methods, including solvent precipitation with acetonitrile, solid-phase extraction (Oasis HLB sorbent and mixed mode sorbent (Bond Elute Certify II)), and liquid-liquid extraction (LLE) (ethyl acetate and methyl tert-butyl ether), three-step LLE with ethyl acetate was chosen as the best method for this application. This LC-MS method was then validated according to FDA guidance. LOQs ranging from 0.1 ng/ml to 0.5 ng/ml were obtained with precision less than 20% and accuracy in the range of 80-120% for all mycotoxins, except for the most polar mycotoxin, nivalenol for which LOQ of 3 ng/ml was achieved. This is the first multi-mycotoxin LC-MS method in human plasma samples that covers multiple mycotoxin classes with sub ng/ml sensitivity.
Low-invasive in vivo solid-phase microextraction (SPME) was used to investigate the metabolomics profiles of muscle tissue in the living fish. In vivo sampling was performed on day 1, 7 and 14. The obtained results were then compared with the metabolomic profiles of fish muscle tissue after one-year storage at -80 °C (ex vivo SPME). Additionally, in vivo SPME extracts were re-run after one-year storage period. Pathway analysis based on Mummichog algorithm was performed on all of the peaks collected from LC-MS in order to evaluate the influences of different sampling strategies. Obtained results showed that in vivo SPME facilitated the collection of 715 peaks, whereas after one-year storage period, only 268 peaks were found in ex vivo SPME fish muscle samples and 562 peaks were found in in vivo SPME extracts. Pathway analysis revealed that in vivo SPME sampling facilitated the extraction of the peaks related to multiple biochemical pathways, including glycosphingolipid metabolism, de novo fatty acid biosynthesis, squalene and cholesterol biosynthesis, propanoate metabolism, N-glycan degradation, glycosphingolipid biosynthesis, glycerophospholipid metabolism, tryptophan metabolism and fatty acid activation (p < 0.01). On the other hand, in ex vivo SPME samples, the peaks affected valine, leucine and isoleucine degradation, butanoate metabolism and glycine, serine, alanine and threonine metabolism (p < 0.01) were found. In re-run in vivo SPME extracts only the peaks involved in the pathway of purine metabolism and tryptophan metabolism (p < 0.01) could be detected. Presented results indicate that in vivo microsampling enabled the identification of different classes of compounds, mainly related to the lipid metabolism and also captured short-lived compounds not detected after one-year sample storage. Long term storage of the muscle tissue as well as SPME extracts might change the metabolomic profiles and cause loss of important compounds or even whole pathways.
Cosmetics containing active pharmaceutical ingredients (APIs), are commercially available, often via the internet, in the US market. These APIs may have physiological effects and have the potential to produce adverse health effects, especially when used without medical supervision. To more effectively monitor products, an ultra high performance liquid chromatography and Q Orbitrap mass spectrometry method, to determine 47 APIs in cosmetic products has been developed. A high-resolution product ion spectral library has been created and used to identify these APIs in a wide variety of cosmetic matrices. The method was validated (single lab) for all 47 APIs in a variety of cosmetic matrices and then applied to the analysis of more than 100 commercially available cosmetic products. Our results show that most of the analytes have linear response in the range of 1 to 1,000 ng/mL with coefficients of determination ($r^2$) greater than 0.99. Further, typical recoveries for the APIs in various matrices ranged from 80-115%. Of the 102 samples tested 4 had APIs not listed on the label and only 8 of the 47 APIs were found in the products.
Glycosylation is a critical quality attribute of protein drugs such as monoclonal antibodies. The traditional assay for glycan profile quantitation involves complex, time-consuming steps including cleaving of the glycans, fluorescent labeling, sample clean-up, and separation. Glycosylation analyses have been too slow for use during production, so a quantitative, high-throughput assay for glycosylation species of monoclonal antibody drugs would be beneficial for monitoring the protein glycosylation profile change during the manufacturing process. We are investigating a streamlined approach where the glycosylated Fc fragment is released from the antibody followed by direct liquid chromatography-mass spectrometry (LC-MS) analysis without labeling or clean-up steps. A key to this advance is a new column for hydrophilic interaction chromatography (HILIC), which has a bonded phase of linear polyacrylamide chains on nonporous silica particles. This polymer allows for intact protein separation without the need for trifluoroacetic acid (TFA), enabling sensitive mass spectrometry. The mobile phase additive for separation and electrospray ionization is difluoroacetic acid. The main glycoforms of a pharmaceutical monoclonal antibody are shown to be resolved by the HILIC column in 5 minutes, as the glycoforms are identified by mass spectrometry. The entire assay time is 20 minutes at the time of this writing. This speed is practical for real-time monitoring of the manufacturing process for monoclonal antibody drugs.

We thank Genentech and the National Institutes of Health (grant number XXXXXXXXXXX-XXXX) for financial support. *** we will update shortly with grant number.
Remote control of media composition during reaction enables to enhance process yield, to improve product quality and to save its time and cost. Fiber spectroscopy allows to run remote process-control in-line with fiber probes immersed directly into reactor and resistant to a harsh process parameters: aggressive, radioactive or toxic chemicals, vibrations, high or low pressure, high or low temperature, etc. The best solution for process-control can be found from experimental trials of the key spectroscopy methods: analysis of media absorption or transmission, diffuse reflection, fluorescence or Raman scattering. Multi-Spectral Fibers (MSF-)System was made to compare these methods advantages and drawbacks using the set of fiber coupled spectrometers for all 4 methods. They all were tested in parallel for the real time tracking of media composition changes during reaction. Comparison of spectral data obtained by all methods enables to select the most accurate one. Possible difficulties in only one method selection can be also resolved by the choice of an optimal combination of 2 or 3 methods united in one combi-fiber probe - to achieve the synergy fusion of the obtained multi-spectral data. Application examples will be presented for MSF-system with a broad spectral range 0.4-16µm, including the “finger-print” part of Mid IR-spectrum covered by IR-fiber coupled FTIR-spectrometers, to demo 2 unique possibilities:

1) use of MSF-System for a fast selection of the best spectral method (or methods combination) derived from experimental tests for reaction monitoring;
2) definition of spectral fiber SF-sensors design from MSF-system trials for dedicated process control. These SF-sensors will possess by low cost and small size as they will use only a few information rich spectral features. Design of customized SF-sensors will match IoT concept - with friendly software, data transfer to iCloud and compatibility with automated production in plants.
Methodology has been developed for the extraction, fractionation, isolation and characterization of radiolabeled metabolites formed in biological tissues. Simple and step-wise procedures are used to isolate metabolites in complex matrices with an ultimate goal of structure identification. These techniques are necessary to satisfy many environmental regulatory requirements. Limits of detection for both the parent analyte and the metabolites can often be in the low ppb range.
Suppressed ion chromatography is an inescapable tool for ion analysis, from analysis of any biological samples (blood, urine, milk) to non-biological samples (tap water), ion chromatography allows the identification and quantification of various anions and cations. Today with the creation of better suppressors and highly efficient columns, the quantitation limit is always challenged and pushed to a lower limit. High resolution separation is a critical factor in Chromatography, but often a feature that is not given too much of an emphasis is quantitation. An inadequate quantitation can easily lead to erroneous results and therefore compromising the integrity of the entire result. We will discuss in this presentation the many ways we can improve quantitation protocols in ion chromatography.

Abstract Text

Suppressed ion chromatography is an inescapable tool for ion analysis, from analysis of any biological samples (blood, urine, milk) to non-biological samples (tap water), ion chromatography allows the identification and quantification of various anions and cations. Today with the creation of better suppressors and highly efficient columns, the quantitation limit is always challenged and pushed to a lower limit. High resolution separation is a critical factor in Chromatography, but often a feature that is not given too much of an emphasis is quantitation. An inadequate quantitation can easily lead to erroneous results and therefore compromising the integrity of the entire result. We will discuss in this presentation the many ways we can improve quantitation protocols in ion chromatography.

Keywords: Calibration, Chromatography, Data Analysis, Quantitative
Application Code: Other
Methodology Code: Process Analytical Techniques
Automated Liquid Handling (ALH) & manual pipetting equipment is an increasingly essential component of life science and chemical laboratories demanding accurate, reproducible and efficient delivery. Used to boost the repeatability of volume transfers, ALH must be optimized to achieve accuracy in liquid delivery. A reproducibility study was conducted with various known methods reporting the specificity, volume range, accuracy, precision, and liquid class settings as well as other environmental parameters. A collaboration with Stanford University, Progenity, Abcam, and Calibrex has been conducted to reproduce results and report which techniques and methods found to be the best and most applicable for various volumes. Systems that were tested were the QC & Service Cal Kits from Automation Trainer LLC, Gravimetric using a precision scale made by Metro Toledo, and the MVC from Artel. To address user needs, we have reviewed various liquid handling quality assurance measurement systems for validating, volume verification/calibration. Various dye based methods in DMSO or aqueous solutions and gravimetric methods. We look at equipment specifications, liquid classes, and volume operating ranges with statistics behind processing and evaluating results. We discuss the lab demand, user pains, success with each method. Significant scientific/market needs and regulatory obligations will be discussed. We shall also look at efficiency and cost effective methods for calibrating equipment in the field for site or factory acceptance. In addition, we investigate how each satisfy user driven needs, compliance and regulatory with NIST traceability, ISO certifications, good practices, techniques, validated methods.

Keywords: Bioanalytical, Genomics, Laboratory Automation, Quality Control
Application Code: Quality/QA/QC
Methodology Code: Process Analytical Techniques
# Process Studies and Improvements

## Quality Control and Calibration Approaches for Determining Reproducible Results

Automated Liquid Handling (ALH) equipment is an increasingly essential component of life science and chemical laboratories. Used to boost the productivity, repeatability of volume transfers, ALH must be optimized to achieve precision and accuracy in liquid delivery. To address user needs, we have reviewed various liquid handling quality assurance measurement systems for validating, volume verification, and calibration. Based on years of practical laboratory experience training thousands of students, we surveyed many of our students and asked which methods they use, prefer, and find most useful. We look at various dye based methods in DMSO and aqueous solutions and gravimetric methods. We shall look at equipment specifications, liquid classes, and volume operating ranges with statistics behind processing and evaluating results. We discuss the lab demand and user pains with each method. Significant scientific/market needs and regulatory obligations will be discussed. We shall present the types of users utilizing ALHs that require calibration presenting statistical data on accuracy and precision: that reveal instrument capabilities to perform actual pipetting within specifications requiring minimal setup time. We shall also look at efficiency and cost effective methods for calibrating equipment in the field for site or factory acceptance. Users as in Forensics need optimum performance out of their equipment to not experience process cost impacts with validated processes for QA/QC assuring proper functionality after every service call, install, move, and operation. In summary, we shall look at satisfying user driven needs, compliances and regulatory with NIST traceability, ISO certifications, good practices, techniques, and validated methods.

**Keywords:** Bioanalytical, Biotechnology, Calibration, Laboratory Automation

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

**Methodology Code:** Process Analytical Techniques
The ability to accurately monitor trace levels of N2O, CO2, CO, THC, and H2O in bulk gases is critical to ensure gas quality and safety. Currently, Non-Dispersive Infrared (NDIR) and Total Hydrocarbon – Flame Ionization Detector (THC – FID) analyzers lead the market for measuring these gases. However, these analyzers require constant calibration to ensure data quality. The MKS MultiGas™ Tunable Filter Spectrometer (TFS™) provides an alternative to the traditional analyzer technologies for trace hydrocarbon, moisture, CO, CO2 and N2O measurements, enabling low cost yet high performance analysis. The analyzer is permanently calibrated, reducing the need for costly reference gas mixtures and does not require fuel mixtures such as those used for THC-FID analyzers. The calibration stability is guaranteed through the use of a first-principle measurement technique and an advanced spectral processing algorithm that compensates for baseline variations. It uses an innovative and patented tunable filter spectroscopy technology enabling highly selective and stable measurements. This measurement technology provides spectral selectivity similar to FTIR analyzers, but with the simplicity and ease of operation of NDIR analyzers. Part per billion detection limits are achieved for most gases through the use of high throughput optics coupled with a long-path gas cell and a high sensitivity detector. Another benefit to the technology is that all of the gases listed above can be measured in a single analyzer, simplifying the sampling handling and data acquisition system as well as the maintenance burden on gas plant operators. Laboratory and field testing has shown exceptional performance and will be presented.

Keywords: Analysis, Instrumentation, Molecular Spectroscopy, Process Monitoring
Application Code: Process Analytical Chemistry
Methodology Code: Molecular Spectroscopy
### Process Studies and Improvements

#### Abstract Title

**Different Methods for Noise Analysis Including 1D, 2D, 3D, and Multi-D Fokker-Plank Equation, Dynamical Programming, and Multi-scale Time Analysis Applied to Reactions Kinetics and Single Molecules Experiments**

#### Primary Author

Michael Fundator  
DBASSE of National Academy of Sciences

**Date:** Wednesday, February 28, 2018  -  Aft  
**Time:**  
**Room:** Exposition Floor, Aisles 2000-2700

#### Abstract Text

Noise models in reactions kinetics evaluated through Kramers model based on Fokker-Plank stochastic differential equation for velocity of chemical reactions and extended from 1D to 2D, 3D, and multi-D Fokker-Plank equation can be applied to noise models, such as Brownian noise caused by the thermal agitation of the molecules of the fluid along with different single molecule experiments, such as optical tweezers, scanned tip microscopes, and single molecule fluorescence methods and can be modeled by Dynamical Programming along with multi scale time analysis.

#### Keywords

- Analysis, Process Control, Single Molecule

#### Application Code

Process Analytical Chemistry

#### Methodology Code

Process Analytical Techniques
Analytical laboratories must begin all their sample analysis by preparing calibration curves. Large volumes of every given concentration are produced and transferred into smaller vials. Each calibration curve aliquot is then stored separately and this tedious process is generally done manually. Using a CERTUS FLEX liquid dispenser, an automated procedure for the preparation of the calibration curve and aliquots is instead established. Three solutions are used: a stock solution of the standard, a dilution solution and a matrix solution. The dispensing system is then set to a specific delivery volume for the preparation of the blank and the 8 calibration standards directly into 2 mL aliquot vials. After the calibration curve preparation, the blank and the standards are extracted and analyzed using an LDTD-MS/MS system.

Three solutions are added on the CERTUS FLEX system: a matrix solution, a dilution solution and a stock solution. A rack containing 54 vials (2 mL) is used as the aliquots container. In each vial, the liquid dispenser system adds 900 µL of matrix as well as 100 µL of different ratios of diluent solution/stock solution. One blank and 8 calibration curve standards are prepared. In a single run, six calibration curves (one blank and 8 standards each) are automatically prepared and aliquoted. Each calibration curve is extracted using a liquid-liquid extraction (LLE) and analyzed using a High Throughput LDTD-MS/MS system.

LLE of the calibration curve aliquot is prepared as follows: 100µL of a sample are mixed with 10 µL of IS solution and 100 µL of buffer. Then, 400 µL of solvent are added, mixed and 2 µL of the supernatant are placed on a LazWell plate and evaporated. Samples are desorbed using a laser power ramp of 0 to 45% in 6 seconds. The mass spectrometer is operated in positive MRM mode. The linearity, slope and accuracy of each calibration curve preparation are evaluated. The interference of the blank at the lowest standard is verified.

Keywords: Bioanalytical, Calibration, High Throughput Chemical Analysis, Sample Handling/Automation
Application Code: Process Analytical Chemistry
Methodology Code: Sampling and Sample Preparation
Photoionization Mass Spectrometry for Catalytic Process Monitoring

Photoionization is a highly effective soft ionization technique, which is characterized by the high yield of molecular ions and very low degree of fragmentation. The coupling of photoionization and mass spectrometry has proven itself as a powerful analytical technique for online and real-time analysis of volatile organic compounds (VOCs) in the fields of environmental pollutant analysis, combustion chemistry studies, and catalytic process monitoring. Methanol to olefins (MTO) is an important industrial catalysis technology for the production of low carbon olefins, the basic chemical raw materials, which has become the key technique in coal to olefins route, and thus it is of great economic value and strategic significance. However, it is a great challenge for traditional analytical methods to real-time and online analyze the catalytic products during MTO catalytic reaction process.

In this study, an online monitoring system based on RF-windowless discharged lamp photoionization time-of-flight mass spectrometry was built for analysis of products during MTO catalytic reaction process. Compared with the commercial low-pressure discharge vacuum ultraviolet (VUV) lamps, such as commonly used 10.6 eV krypton (Kr) lamp, the windowless VUV lamp have higher photon flux, better long-term stability, and allows adjusting the photon energy by changing the gas filling. As a result, a mass resolution of 3960 was achieved at m/z 28, and the mass peaks of N2 and C2H4 were baseline separated. The limits of detection down to 83.8, 53.6, and 39.8 pptv were obtained for ethylene, propylene and butylene within 5 s, respectively. And the RSD is inferior to 1.19% in eleven hours during continuous monitoring. The instrument has been successfully applied to the real-time, fast, and online analysis of gas-phase products during MTO catalytic process. The results indicate that this instrument had potential application value and broad application prospects in industrial process monitoring.

Keywords: Detection, On-line, Process Monitoring, Time of Flight MS
Application Code: Process Analytical Chemistry
Methodology Code: Mass Spectrometry
Discussion of Energy Dispersive X-ray (EDXRF) Technology's Application to Measure the Absolute Fuel Parameter of Gasoline Sulfur to Comply with the Tier 3 Gasoline Standards

The adoption of the new Tier 3 Program by the Environmental Protection Agency (EPA) has resulted in the need for increased sensitivity in instruments to meet the updated testing requirements. The new program has mandated revised regulations on the composition of fuel, scaling down the maximum allowable sulfur content to 10 parts per million (ppm). The EPA considers vehicles, and the fuel it uses, as a major contributor of adverse effects to the environment and to public health. It is predicted that 10% to 30% of air toxins will be mitigated due to the new policy, this will improve both the environment and overall public health.

Moreover, the burden of oversight on monitoring the sulfur content in fuels has been placed on global field refiners, and it is their incentive to be confident that the reported data to the EPA is reliable and accurate. The EPA accepts the ASTM D7220: “Standard Test Method for Sulfur in Automotive, Heating, and Jet Fuels by Monochromatic Energy Dispersive X-ray Fluorescence Spectrometry,” to verify the absolute fuel parameter of gasoline sulfur. This poster discusses a new benchtop analyzer which is a simple, compact, and versatile analytical tool that utilizes polarized X-ray technology to accurately determine the sulfur content of samples in question, and presents experimental data with related statistics for a variety of samples. This unique analyzer has the necessary precision and accuracy to meet the requirements for testing set by the EPA Tier 3 program for ultra-low sulfur fuels such as gasoline and diesel, while complying with ASTM D7220.

Keywords: Environmental, Fuels\Energy\Petrochemical, X-ray Fluorescence
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Atomic Spectroscopy/Elemental Analysis
The rapid development of drug discovery has pushed high demand for measuring real time binding kinetics for proteins of interest and small-molecular-weight inhibitors without labeling and tethering. Traditional methods usually rely on labeling, tethering, or fluorescence which affect conformation and activity of the proteins; also, these methods analyze the average performance of a large ensemble of proteins. Our work focuses on trapping single proteins of interest while keeping the binding sites and conformation unchanged. Single proteins are trapped using a double nanohole (DNH) nanoaperture, which is fabricated into a 100nm thick gold film. This type of optical trap doesn’t rely on the gradient force but on the self-induced back-action (SIBA) effect of a trapped dipole in an optical resonator. The intensity of the transmission signal is sensitive to the polarizability of the trapped protein. And the polarizability of the protein changes as it changes confirmation. Time resolution of this single molecule approach is at sub-nanosecond scale which indicates a more precise and rigorous real-time binding kinetics between the protein of interest and a small molecule without any ensemble averaging effects. High-affinity binding complexes, biotin-streptavidin, observed through DNH have different behaviors from glucose-glucokinase or, low affinity binding complexes. Moreover, glucokinase has one single glucose binding site but a high conformation change which indicates further glucose binding and product release. This allosteric interaction can also be observed. Further development of this single protein trapping technique has a promising future in analytical chemistry.
Photo-ionization time of flight mass-spectrometry (PI-TOFMS) is well suited for on-line characterization of cigarette smoke, tobacco heating products, joints and e-cigarettes. Depending on the PI method (single photon [SPI] or resonance-enhanced multiphoton ionization [REMPI]) compounds such as butadiene, acetaldehyde, naphthalene, phenol or PAHs can be detected with high time resolution. Within the last years e-cigarettes and other new smoking/vaping products became more and more commonly used. An on-line puff by puff analysis of thermal degradation products has the challenge to enable an analyte separation without pre-separation although the starting point of the thermal decomposition is a complex mixture and will provide multiple reaction pathways. With the increasing legal availability not only for medical purposes of marihuana/cannabis and THC (Tetrahydrocannabinol) containing smoking products in various countries the interest in understanding the release processes of the active components is increasing as well. Puff by puff analysis of different joints filled with tobacco mixtures containing dried marihuana flowers, leafs or hashish were performed to investigate the release profile of THC and related compound in comparison to nicotine as the main active compound of the added tobacco. Within this study a Laser PI-TOF system (Photonion GmbH, Germany) was used coupled to a LM1 smoking machine (Borgwaldt KC, Germany). Especially the REMPI methodology enables focusing on aromatic structures. Smaller molecules being present in higher amounts (e.g. aldehydes), being able to influence the measurement of the active compounds, are suppressed effectively. Environmental gases such as oxygen or nitrogen are suppressed by photoionization anyway. Furthermore, the present study evaluates the influence of activated carbon filters becoming more and more popular to reduce the negative health effects of smoke compounds such as PAHs especially in smoking of THC containing products.

Keywords: Drugs, Instrumentation, Mass Spectrometry, Method Development
Application Code: General Interest
Methodology Code: Process Analytical Techniques
**Session Title:** Process Studies and Improvements  
**Abstract Title:** Real-Time Monitoring of Uranium (VI) in Nuclear Materials Reprocessing  

At the Savannah River Site, improvements in the efficiency of processing spent nuclear fuel bundles are being sought through several means, including more widespread implementation of real-time monitoring of actinide concentrations in the process streams. Process throughput is highly limited by the turn-around time of offline analyses. We are applying recent instrumental and chemometric advances to the measurements of uranium and nitric acid concentrations in our facilities. Here, we will describe improved sensitivity and precision of U(VI) measurements through the use of a blue LED-enhanced continuum light source for higher signal quality near the U(VI) absorption bands. A co-added atomic line source allows for continuous wavelength calibration and supports effective calibration transfer between instruments without use of a transfer matrix. We will also address the use of piecewise local partial least squares (PLS) modeling to improve measurement robustness by reducing the overfitting that can occur with global PLS models that are defined over a widely varying calibration space. These developments will lead to implementation of these monitors at more challenging locations in the process, for example in streams where substantial interferents will be present from the dissolution of the bundles, and decrease the number of offline analyses that must be performed.

**Keywords:** Chemometrics, Nuclear Analytical Applications, Process Analytical Chemistry, UV-VIS Absorbance/Lu

**Application Code:** Nuclear

**Methodology Code:** Process Analytical Techniques

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**Primary Author:** Robert Lascola  
Savannah River National Laboratory  

**Co-Author(s):** Agnerys Rodriguez-Santos, David Immel, Jean Plummer, Patrick E. O'Rourke  

**Date:** Wednesday, February 28, 2018 - Aft  
**Time:**  
**Room:** Exposition Floor, Aisles 2000-2700
Inositol-1,2,3,4,5,6-hexakisphosphate (IP6) is a natural product of plant tissue and one of the most common natural organophosphates found in environmental systems. Overall, IP6 is highly stable under natural conditions and is generally only degraded through a select few enzymatic pathways. This abundance and stability in soil and aquatic systems makes it a largely untapped reservoir of bioavailable orthophosphate for use by organisms and plants, particularly in agriculture. As such, it is important to be able to identify and quantify the amount of IP6 and its dephosphorylated congeners, IP5-1, found in media such as soil, sediment, surface water, and groundwater. In this study, we will present methodology for the isolation, identification, and quantification of IP6 and subsequent congeners that have been enzymatically dephosphorylated using wheat phytase enzymes. A novel solid phase extraction technique has been developed for the isolation of phytate from environmental media including water, soils, and sediments. Quantification of IP6 and congeners has been conducted using inductively coupled plasma mass spectrometry (ICP-MS) and high performance liquid chromatography with UV-Vis detection (HPLC-UV) following derivatization. Structures of the lower order inositol phosphates found in the environmental samples, as well as those obtained by the enzymatic degradation, were characterized using proton and 31P nuclear magnetic resonance spectroscopy (NMR).

**Keywords:** Liquid Chromatography, Solid Phase Extraction, Environmental Analysis, ICP-MS

**Application Code:** Environmental

**Methodology Code:** Separation Sciences
Since metal-bound (holo) metalloproteins are involved in various biological processes, the identification of holo-metalloproteins in biological samples is of importance. However, there is no general method specific to isolate and identify holo-metalloproteins in complex biological samples without several separation steps. In our previous work, it was reported that metal ion contaminant sweeping-blue native-polyacrylamide gel electrophoresis (MICS-BN-PAGE) was successfully developed for effective protein separation without serious contaminant metal ions.[sup]1[/sup] In that work, it was unexpectedly found that electrophoretic mobilities of metalloproteins were significantly different between holo- and apo (metal-dissociated)-forms.

In this study, we developed a novel isolation method selective to holo-metalloprotein (called holo/apo conversion-2D MICS-BN-PAGE) via chemical conversion from holo- to apo-form between the first and the second MICS-BN-PAGE by soaking in acidic solution. In the second PAGE, holo-metalloproteins in the original sample, which are converted into apo-forms, migrate to different distances from those of the first PAGE. Thus, only holo-metalloproteins in the samples are feasibly isolated as spots off of the diagonal line.

We applied this method to a periplasm sample of [i]Rubrivivax gelatinosus[/i], which is a kind of purple bacteria. While it has already been found that CopI proteins expressed with the addition of Cu[sup]2+[/sup] ions to a [i]Rg[/i] sample,[sup]2[/sup] it was obscure whether Cu[sup]2+[/sup] ions bound to CopI. Using holo/apo conversion-2D MICS-BN-PAGE, a Cu-metalloprotein was successfully isolated. The isolated Cu-metalloprotein was identified as CopI by MALDI-TOF MS. Thus, it was revealed that Cu[sup]2+[/sup] ions in the periplasm space of [i]Rg[/i] exist as Cu-CopI complexes.


Keywords: Bioanalytical, Electrophoresis, Proteomics, Speciation
Application Code: Bioanalytical
Methodology Code: Separation Sciences
The determination of aliphatic/aromatic compounds when performing Total Petroleum Hydrocarbon (TPH) analysis is typically a lengthy process – with solid phase extraction (SPE) to separate aliphatics into hexane and aromatics into dichloromethane, prior to two separate GC analyses per sample.

The enhanced separation of comprehensive two-dimensional chromatography (GC×GC) negates the requirement for laborious sample fractionation, saving a significant amount of extraction and processing time, while also reducing consumable costs. Instead of two separate analyses, the traditional ‘boiling point’ separation is maintained in the first dimension while the aliphatic and aromatic compounds are separated in the second dimension.

Fast and confident group-type quantitation is then performed, using simple data processing tools. Stencils are applied to group peaks into chemical classes (e.g. C8-C10, C10-C14 etc) based on their elution region. The use of flow rather than thermal modulation, ensures excellent retention time repeatability across large sample batches, enabling automated data processing to be performed with minimal operator intervention.

Furthermore, here we demonstrate a dual-channel configuration, doubling the sample throughput per instrument - increasing productivity, reducing bench space and lowering installation costs, all while meeting the criteria set out in standard methods (e.g. TPH Criteria Working Group and Massachusetts State).

**Keywords:** Environmental Analysis, Environmental/Soils, GC, Petroleum

**Application Code:** Environmental

**Methodology Code:** Separation Sciences
A new method was developed for the extraction and spectrophotometric determination of lanthanum(III) using o-methoxy phenylthiourea (OMePT) as a chromogenic ligand. The basis of the proposed method is lanthanum(III)-OMePT complex formation in 0.1 mol L\(^{-1}\) aqueous hydrochloric acid media with 2 mL of 0.01 mol L\(^{-1}\) OMePT in ethanol at room temperature. Lanthanum(III)-OMePT complex was extracted in 10 mL chloroform after single extraction and it was measured at 320 nm against the reagent blank. Beer’s law was obeyed up to 45.0 µg mL\(^{-1}\) for lanthanum. The molar absorptivity and Sandell’s sensitivity of the complex were 1.5002 \(\times\) 10\(^3\) L mol\(^{-1}\) cm\(^{-1}\) and 0.0926 µg cm\(^{-2}\) respectively. The stoichiometry of lanthanum(III)-OMePT complex was 1:1. The stability of lanthanum(III)-OMePT complex was >48 h. Proposed method was successfully applied for determination of lanthanum(III) from binary synthetic mixtures and real samples. Sequential separation of lanthanum(III), Cerium(IV) and Thorium(IV) was achieved.
Propolis is the generic name for strongly adhesive mixture made of bee secretions and plant resins, collected by Stingless bees and honeybees from a variety of plant sources. Propolis exhibit a wide spectrum of pharmacological activities that vary depending on the geographical location, climate and availability of botanical sources for the bees to forage. Because of its popularity in folk medicine, it has become the subject of intense pharmacological and chemical studies over the last 40 years. Increased use of propolis preparations necessitates analysis of its composition and identification of its pharmacologically active constituents. The aim of this research was focused in the evaluation and use of simple, selective, and sensitive HPTLC method for study and classification 53 samples of bee propolis collected at different biogeographical wildlife zones in Colombia. Chromatographic analysis was performed on 20 cm × 10 cm glass-backed silica gel F 254s HPTLC plates with Toluene-Acetyl acetate-formic acid, 44:3.5:2.5 (v/v) as mobile phase. Quantification was performed by scanning densitometry at the UV absorption maximum of each compound. The methodology was found to be simple, reliable, selective and linear and convenient for analysis of phenolic compounds. This work is a contribution to the study and knowledge of the colombian propolis and allows the separation and identification of its components due to analytical performance respect to precision, accuracy and robustness, fulfilled the acceptance criteria established for TLC methods in the official literature.
Silica-based stationary phases have long been the workhorse of modern liquid chromatography. However, silica has disadvantages in the separation of some ionic and polar analytes, and it has stability issues at both high and low pH. These concerns have led various analytical chemists to explore novel stationary phases beyond silica. Carbon particles, including porous graphitic carbon (PGC), have previously been explored as LC stationary phases. They offer superior pH stability compared to silica-based stationary phases. Carbon-based stationary phases, including graphitic carbon columns, e.g., HypercarbTM, can separate structurally similar compounds and are suitable for high temperature separations. The retention mechanism for this phase is often referred to as a polar retention effect on graphite (PREG). Here, solutes with polar character are retained because of image charge effects in the graphitic stationary phase. However, one of the significant issues associated with porous graphitic carbon is analyte tailing. In this presentation we describe the derivatization of graphitic carbon particles with a series of reagents and their subsequent surface/material characterization. This chemical modification is designed to create a phase with minimal tailing. We demonstrate the packing of these particles into capillary columns and show separations on them, comparing the resulting separations to those we obtain from unfunctionalized carbon.

Keywords: Capillary LC, Derivatization, Liquid Chromatography, Surface Analysis
Application Code: Process Analytical Chemistry
Methodology Code: Surface Analysis/Imaging
Modification of Silica Particles with Nanodiamonds Primary Particles for Liquid Chromatography

One of the most investigated aspects in high performance liquid chromatography (HPLC) is column technology. This is the case as new stationary phases are explored to enhance selectivity, high efficiency, and/or faster analysis times. Silica is the most common support material used in HPLC due to its physicochemical characteristics and to the simple surface derivatization techniques available. We are investigating the surface modification of silica particles with nanodiamonds and its potential use in HPLC. We have modified the surface of silica particles with an amino-containing silane that acts as an anchor for the spontaneous grafting of commercially available nanodiamonds (NDs) onto the silica surface. The nanodiamond particles contain various functional groups on the surface that can act as adsorptive sites for HPLC and also provide a means for further functionalization. The particles have been characterized by infrared spectroscopy (IR) and electron transmission microscopy (TEM). Details of the derivatization process and the physicochemical characterization of the ND-silica materials will be the focus of this presentation.

Keywords: HPLC, Materials Characterization, Modified Silica
Application Code: Material Science
Methodology Code: Liquid Chromatography
## Abstract Text

There has been an increasing importance of the development of rapid Sr separation techniques for Sr-90 analysis, responding to needs in Fukushima Daiichi NPP. However, conventional Sr-90 analytical methods require subsequent separation of Sr and Y, resulting in a long processing time of about one month. We are trying to realize a rapid analytical method using a Sr separation material which adsorbs only Sr and enables beta-ray measurement with adsorbed state. In this study, we prepared the Sr Fiber that has a high-density Sr adsorption phase on its surface. The Sr Fiber adsorbs Sr-90 only onto the fiber surface, allowing to highly efficient beta-ray counting by minimizing the self-attenuation effects. The adsorption capacity of the prepared Sr Fiber was about 14 g/mol, which is equivalent to that of a commercially available Sr adsorptive resin (Sr Resin). The selectivity of the Sr Fiber was nearly the same as that of the Sr Resin. Considering that the Sr Fiber has a specific surface area 1000 times smaller than that of the Sr Resin, the Sr ions can be concentrated to 1000 times on its surface, capable of achieving highly-efficient beta-ray counting. From these result, we confirmed that Sr Fiber has enough adsorption capacity and selectivity for efficient beta-ray counting of Sr-90.

**Keywords:** Extraction, Method Development, Radiochemical Methods

**Application Code:** Process Analytical Chemistry

**Methodology Code:** Separation Sciences
Functional nanoparticles have become important parts of nanotechnology and been widely used for various applications on material science and biochemical analysis. Multifunctional nanoparticles have great potential to be used in many new applications. Magnetic immunoassay is a relatively new and effective technique for bioanalysis. This presentation would show several biochemical analyses using functional nanoparticles and magnetic immunoassay in thin channels. Model biomarkers would be used to demonstrate the applications of this technique. In comparison with other methods, this method has lower detection limit and wider linear range. This technique has great potential to provide a simple, fast, sensitive, and selective analysis for particles, proteins, and other biomaterials.
Ambiguous media coverage about the pros and cons of “nano-enhanced” consumer products in recent years has significantly propelled the discussion about their safety. With this ongoing discussion, regulatory authorities such as the European Commission launched several regulations dealing with the declaration of products, which contain nanomaterial ingredients. One is the “European Regulation EC No 12232009 of 30 November 2009 on cosmetic products, in which it is stated that “all ingredients present in the form of nanomaterials shall be clearly indicated in the list of ingredients. The names of such ingredients shall be followed by the word ‘nano’ in brackets”. However, until now, there is still a clear lack of available analytical methodologies, which can provide a straightforward and reliable testing procedure for such products.

We herein present a novel approach to reliably assess the nanoparticulate content of commercially available sunscreens. This approach encompasses a mild and environmentally friendly removal of water and lipophilic sunscreen ingredients via inverse supercritical carbon dioxide extraction (scCO2) followed by the determination of the size distribution as well as the elemental composition of the nanoparticulate content via miniaturized Asymmetrical Flow Field-Flow Fractionation hyphenated with Multi-Angle Light Scattering and Inductively-coupled Plasma Mass Spectrometry (mAF4-MALS-ICP-MS).

This setup enables a straightforward and clear distinction of “non-nano sunscreens” from “nano sunscreens” with high confidence under environmentally friendly conditions and has the potential to be the testing procedure of choice, when it comes to the verification of the “nano-labelling” of commercially available cosmetic products.
The determination of detection and quantification limits (LOD and LOQ) is essential to good method validation. This poster describes how a precise and versatile autosampler (ALS) program method may be used for estimating LOD/LOQ. Typically, an analyte may require many dilution preparations to determine the smallest concentration that can be reliably measured. The ALS is used to automate solvent dilution tasks to systematically investigate concentration ranges near LOD/LOQ limits. The ALS programming was employed to perform dilutions for estimating the LOD/LOQ for the method development determination of Caffeine and Quercetin. The technique streamlined the signal-to-noise ratio and visual approaches to estimating LOD/LOQ determination with excellent linear regression results.
Antibody-drug conjugates (ADCs) are promising state-of-the-art biopharmaceutical drugs for selective drug-delivery applications and treatment of diseases such as cancer. ADCs consist of drug delivering monoclonal antibodies linked to cytotoxic payload molecules. Problems associated with the removal of hydrophobic cytotoxic compounds from the intracellular environment have driven the development of novel hydrophilic payload molecules, without losing the essential cytotoxic ability of the drugs. In order to assess the hydrophobicity of novel ADC payload drugs capillary electrophoresis, and particularly electrokinetic chromatography, were utilized. In electrokinetic chromatography the hydrophobicity is estimated based on analyte partitioning between an aqueous phase and a pseudostationary phase. ADC payload drugs are known to affect the cell mortality intracellularly but they could not interact with standard biomimetic phospholipid vesicles (liposomes), used as a hydrophobic pseudostationary phase in electrokinetic chromatography. This data indicates that the compounds were not able penetrate the liposome membrane. Therefore, the outermost goal of this study was to determine how the interactions between the payload molecules and the pseudostationary phase are influenced by the shape, size, and composition of the surfactant aggregates utilized as a pseudostationary phase.

The pseudostationary phase was composed of neat phosphatidylcholines of different acyl chain lengths, neat negatively charged surfactants, and mixtures of these. The lipid-surfactant mixture ratios were selected based on their different coalescent behavior; a high concentration of phospholipids yield negatively charged vesicles, while high concentrations of surfactants yield negatively charged micelles. From the data we were able to extract valuable information on the hydrophobicity of the studied ADC payload molecules.

**Abstract Text**

Antibody-drug conjugates (ADCs) are promising state-of-the-art biopharmaceutical drugs for selective drug-delivery applications and treatment of diseases such as cancer. ADCs consist of drug delivering monoclonal antibodies linked to cytotoxic payload molecules. Problems associated with the removal of hydrophobic cytotoxic compounds from the intracellular environment have driven the development of novel hydrophilic payload molecules, without losing the essential cytotoxic ability of the drugs. In order to assess the hydrophobicity of novel ADC payload drugs capillary electrophoresis, and particularly electrokinetic chromatography, were utilized. In electrokinetic chromatography the hydrophobicity is estimated based on analyte partitioning between an aqueous phase and a pseudostationary phase. ADC payload drugs are known to affect the cell mortality intracellularly but they could not interact with standard biomimetic phospholipid vesicles (liposomes), used as a hydrophobic pseudostationary phase in electrokinetic chromatography. This data indicates that the compounds were not able penetrate the liposome membrane. Therefore, the outermost goal of this study was to determine how the interactions between the payload molecules and the pseudostationary phase are influenced by the shape, size, and composition of the surfactant aggregates utilized as a pseudostationary phase.

The pseudostationary phase was composed of neat phosphatidylcholines of different acyl chain lengths, neat negatively charged surfactants, and mixtures of these. The lipid-surfactant mixture ratios were selected based on their different coalescent behavior; a high concentration of phospholipids yield negatively charged vesicles, while high concentrations of surfactants yield negatively charged micelles. From the data we were able to extract valuable information on the hydrophobicity of the studied ADC payload molecules.

**Keywords:** Capillary Electrophoresis, Drug Discovery

**Application Code:** Drug Discovery

**Methodology Code:** Separation Sciences
In most industries where analytical work is completed, it is beneficial for diagnostics to be inexpensive and time efficient. Microfluidic methods have been used for chemical and biological analysis, and are gaining quick acceptance into the engineering and technological industries as they allow for fast, relatively immediate diagnosis, especially in limited resource environments. The miniaturized size of the device runs smaller samples, allows for relatively quick analysis and portability, which reduces cost. The work presented here uses paper-based microfluidic analytical devices produced by using Whatman Cellulose Chromatography Paper and a Xerox ColorQube 8580N Color Solid Wax Ink Printer. Methods following the device design done by Gross, E. M., et al. for fabricating reagents, stock solutions, and standard solutions were used to run a quality control check for glucose, protein and nitrites. This work will focus on analyzing for metals in water tributaries around the St. Louis region. Depending on the colorimetric analysis in the test zones compared to standards, it can be determined if there needs to be remediation due to contamination. Analyses will be done on site and in the lab; reproducibility will be tested using visual and spectroscopic methods using image processing or possibly other simple spectrophotometric methods.

Characterization of a Microspectrophotometer for Quantitative Bio-Applications

Spectrophotometers are a universal tool for biological research as they provide a way to quantify and qualify biological materials of interest including DNA, RNA, NADH, proteins, cell density, and other small molecules. However, limitations of a traditional spectrophotometer such as the size, and the cost of the instrument limit its functionality. To improve efficiency in these analyses, the capabilities of a spectrophotometer have been incorporated into the barrel of a micropipette. This microspectrophotometer has several benefits over traditional instrumentation such as relatively low manufacturing costs, small size, mobile, requires very little sample and utility in enclosed or outdoor environments. This instrument utilizes disposable tips like a traditional micropipette. It can be used or even dedicated to clean environments including a PCR/ RNA clean areas, glove bag, BSL3 hood, fume hood, or taken into the field. Data can be viewed on the device or sent wirelessly to a server, computer, tablet, phone, or wearable device. In combination, the benefits of this type of instrument make spectrophotometric analysis much more user friendly and efficient. The newest version of this instrument will be presented highlighting a true beta device with data being reported on that unit. Initial characterization of the device will be done using food dyes, and calibration curves will be completed for DNA and RNA quantifications and NADH determinations.

Keywords: Nucleic Acids, Protein, Spectrophotometry, Spectroscopy
Application Code: Bioanalytical
Methodology Code: Molecular Spectroscopy
This project aims to develop a quantitative paper-based microfluidic device (microPAD) that is a reliable, cost-effective diagnostic tool ideal for use in a home and healthcare settings. This novel 3D microPAD relies on time-of-passage through a degradable metastable biomatrix, as a means for the quantification of a biological analyte. Our device design is incorporated onto stacked Whatman® chromatography paper via wax printing, forming hydrophobic channels that direct sample through target regions on each layer. A metastable gelatin biomatrix blocks passage of a sample through the 3D device, unless the target analyte is present in detectable concentrations, upon which the biomatrix degrades and time of passage through the biomatrix can be recorded. An off-device immuno-selection of a biological sample containing an analyte of interest results in the concentration dependent release of an antibody-Enteropeptidase conjugate. This conjugate is placed on-device and directed toward a pre-spotted zymogen resulting in an enzymatic amplification that leads to the degradation of the metastable biomatrix. The degradation of the biomatrix is analyte concentration dependent, sensitive down to a 459 pM concentration, with higher concentrations correlating to a faster readout time. The devices remain functional at a temperature range of 17-29°C, a room humidity of up to 80%, and sample pH ranging from 1-9. It was determined that the stability of the biomatrix was maintained during storage at room temperature and -20°C for up to three months, however function of pre-spotted enzyme was lost after this period of time. Future direction includes the addition of an on-device digital read out that measures a change in resistance across the biomatrix. With further experimentation this device has the potential for detecting a myriad of alternative serum and salivary biomarkers which could impact global health care.

Keywords: Bioanalytical, Biomedical, Paper/Pulp, Quantitative
Application Code: Biomedical
Methodology Code: Microfluidics/Lab-on-a-Chip
In a variety of possible crimes, identifying different types of paper through trace metal composition can be beneficial to the case and cost effective by using Inductively Coupled Plasma-Optical Emission Spectroscopy. Metal analyses are most commonly done using Inductively Coupled Plasma-Mass Spectrometry, which is extremely sensitive due to the way the analyte is detected in the instrument, but has an inherent cost due to this type of detection. The authors are suggesting using ICP-OES as an alternative way to analyze for trace metals in paper. A concern is this method is that it is not the “go to” instrument that is typically used to identify and quantify metals due to the characteristically higher limits of detection than when compared to ICP-MS, but should be acceptable alternative in this instance. The work presented here was done using the sample preparation method from an ICP-MS analysis by Gh. Tanase, et al., and the digested samples were then analyzed for 12 metals of interest using a Thermo iCAP6500 Duo ICP-OES. The expected outcomes are that the ICP-OES will prove that different types of paper have different amounts of trace metals in them and will also reduce cost per analysis. This data can then be used in identification of papers during different cases where matching paper to a suspect could be critical, to include possible crimes such as kidnappings, murders and counterfeiting documents and paper currency.

Keywords: Atomic Emission Spectroscopy, Atomic Spectroscopy, Forensics, ICP
Application Code: Homeland Security/Forensics
Methodology Code: Atomic Spectroscopy/Elemental Analysis
Dopamine (DA) release in the brain occurs over phasic and tonic timescales. To fully understand DA neurotransmission, it is necessary to develop sensors capable of measuring both tonic and phasic DA profiles. Fast-scan cyclic voltammetry is a widely used electroanalytical technique capable of measuring phasic DA concentration changes, but its inherent limitations prevent the recording of tonic, resting-level DA. Thus, the need remains for a functional system enabling in vivo tonic dopamine detection. We have developed a novel detection system comprising a carbon fiber microelectrode (CFE) incorporating a poly-3,4 dioxythiophene (PEDOT)/functionalized carbon nanotube (fCNT) nanocomposite capable of directly measuring tonic resting DA with high sensitivity and selectivity using a customized square wave voltammetry (SWV) waveform. In vitro sensor development and optimization was conducted through exploring the effects of PEDOT/fCNT nanocomposite thickness and SWV waveform modifications on DA detection sensitivity and selectivity over a primary in vivo interferent, ascorbic acid (AA). Increasing PEDOT/fCNT thickness directly improved DA sensitivity by decreasing CFE impedance, which facilitates electron transfer between DA and the electrode during DA redox events, and enhancing DA adsorption to the electrode surface through electrostatic attraction. PEDOT/fCNT also improved detection selectivity over AA through electrostatic repulsion of AA. Selectivity was further improved by introducing a constant 0V section in the voltammetric waveform before SWV is applied. This causes AA oxidation, decreasing its abundance at the electrode surface and thus minimizing AA presence during DA detection via SWV. With increasing 0V hold times, the AA redox signal was attenuated, and selectivity was thus improved. This sensor has been validated in vitro, and will be tested in vivo for measurement of tonic DA in the dorsal striatum of anesthetized rats.

Keywords: Chemometrics, Electrochemistry, Sensors, Voltammetry
Application Code: Neurochemistry
Methodology Code: Chemometrics
Catecholamines are neurotransmitters that have been suggested to be important in neurodegenerative disorders, such as Parkinson’s disease, including dopamine, epinephrine, norepinephrine, and serotonin. To better understand their role in neurodegeneration, good analytical methods and animal models are necessary. To help accomplish this goal, a method was developed using microchip electrophoresis with amperometric detection to separate and quantify these neurotransmitters. A 5 cm long simple ‘t’ design polydimethylsiloxane microchip that is reversibly sealed to a glass substrate fabricated with a pyrolyzed photoresist film working electrode was used in these studies. The separation was performed with a field strength of 222 V/cm and 1 s gated injection is used for the sample injection. A potential of +1 V (vs Ag/AgCl reference electrode) was applied to the working electrode and was previously determined by cyclic voltammetry.

Several background electrolytes were investigated with concentrations of sodium dodecyl sulfate (SDS) ranging from 2 mM to 20 mM added to the 15 mM phosphate at pH 7.4. A baseline separation of the standards was achieved with 15 mM SDS and the analytes were separated in less than 80 s. The current limits of detection for the dopamine and norepinephrine standards were 500 nM and 1 \( \mu \)M for epinephrine and serotonin. The external standard calibration curve showed good linearity in the 2 \( \mu \)M to 20 \( \mu \)M range. Zebrafish have been used as an animal model for neurodegenerative diseases and results using this method for the analysis of zebrafish brain samples is in progress.

**Keywords:** Biosensors, Electrochemistry, Electrophoresis, Neurochemistry

**Application Code:** Bioanalytical

**Methodology Code:** Microfluidics/Lab-on-a-Chip
Sulf 1, as the 6-O endosulfatase of heparan sulfate proteoglycans (HSPGs), has been reported in many previous works that it plays the critical role in the pathogenesis of a variety of human cancers, especially breast cancer. However, there are very few reports about point-of-care detection of sulf 1 currently. In this work, an electrochemical method using HSPGs as a substrate was employed to detect the concentration of sulf 1. HSPGs, a glycoprotein with the common characteristic of containing one or more covalently attached heparan sulfate (HS) chains and is also a type of glycosaminoglycan, can mediate the activation of a variety of cell growth factor signal pathways to promote the progression of cancers. The disaccharide units of HS chains have four different sulfation modification sites, which are substituted on the N-, 3-O, and 6-O positions of glucosamine and the 2-O position of uronic acid residues. Considering sulf 1 can transform the 6-O sulfation of HSPGs to OH group, as shown in figure 1, the structure and electrical property of HSPGs could take some changes. Here, electrochemical impedance spectroscopy (EIS) was used to detect the changes of electrical properties of HSPGs before and after reacting with sulf 1 standard solution. Through analyzing and calculating the electrical parameters obtained by EIS, such as charge, capacity and degree of phase, we established the calibration curve of sulf 1 concentration, which could be further used to detect the sulf 1 concentration within human blood samples. This work offers a new method for sulf 1 detection and has great potential in the early detection of cancers.
Endocrine disrupting compounds (EDCs) are chemicals that can block or mimic natural hormones in the endocrine system. EDCs enter into the environment through the disposal of unwanted drugs through garbage and after their intended use, through excretion. EDCs are resistant to biodegradation and are highly water soluble, leading to limited removal by wastewater treatment plants. As a result, the compounds can easily enter the aquatic environment biologically active and risk exposure to aquatic organisms’ behavior, anatomy, and physiology. However, there is not sufficient research on the effects of EDCs on aquatic vertebrates to have a full understanding of the damage done to these organisms by EDC pollution. Exposing fathead minnows at environmentally relevant concentrations allows for an accurate representation of how EDCs affect and accumulate in aquatic vertebrates. Mimicking singular exposures for seven EDCs at seven days will represent how EDC pollution affects aquatic wildlife. After exposure the minnows will be homogenized for quantitative analysis by LCMS, or used for qualitative analysis by MALDI MSI. Imaging the minnows determines precisely where these pharmaceuticals accumulate because each EDC used could affect separate tissues and cause distinctive side effects. The results show after seven days of exposures there is accumulation of EDCs in regions of fathead minnows.
With new various chemicals in society, research is needed to detect and assess various trace organic compounds (TOrCs) in the environment. Polybrominated Diphenyl Ethers (PBDEs) are contaminants that have been added to many plastics to act as a flame retardant by suppressing the combustion process. As a result of product disposal, these chemicals have leached into the environment and have been detected in biota. This can be a problem for the ecosystem because some are nearly nondegradable, and can bioaccumulate. To date, the environmental fate and transport of these compounds is not fully understood. In this work, we sought to measure the octanol-water partition coefficients of a select subset. Their extreme hydrophobicity makes analysis a challenge and first steps involved optimization of a liquid-liquid extraction procedure. Gas chromatography-triple quad-mass spectrometry was employed for separation and detection. The log Kow values of this select subset of PBDEs were determined by the Slow Stirring Method. These values, coupled with other properties and toxicity data, will allow a more complete understanding of the fate and transport of these compounds, leading to a better assessment of their risk to environmental and human health.


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Undergraduate Poster Session

**Abstract Title**: Analysis of Melamine in Pet Food with Gold Nanoparticles and UV-Vis Spectroscopy

**Primary Author**: Elizabeth Goodrow  
**Washburn University**

**Co-Author(s)**: Aaron Hummert, Seid Adem

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**Abstract Text**

The purpose of this research is to develop a colorimetric sensor using gold nanoparticles (GNPs) to detect and analyze the presence of melamine in solid pet food samples. Due to its low cost and high nitrogen content, there has been evidence that melamine has been illegally added to various products, such as pet food and infant formula, to falsely increase the apparent protein content. The currently used methods, such as liquid chromatography/mass spectrometry (LC/MS) and capillary electrophoresis/mass spectrometry (CE/MS), are expensive, time-consuming, and require skilled personnel. Thus, there is a need to develop cheap, fast, and portable technique to detect and analyze melamine contamination.

Techniques based on gold nanoparticles are being developed for this purpose. When GNPs are in their colloidal state they exhibit a wine-red color and become blue or purple when they aggregate in the presence of melamine. This immediate color change is particularly useful when portable detection is required. The aggregation-based change in color can also be monitored through the use of UV-Vis spectroscopy. In the presence of melamine, the absorption band of GNPs shifts from 520 nm to above 750 nm. The limit of detection for this method was determined to be 0.12 ppm. Interference study was conducted to evaluate the selectivity of the technique using many substances found in pet foods. Only melamine caused a color change and a shift to longer wavelength of the absorption band of the gold nanoparticles, indicating the selectivity of the technique for melamine detection.

**Keywords**: Food Contaminants, Nanotechnology, Sensors, UV-VIS Absorbance/Luminescence

**Application Code**: Food Safety

**Methodology Code**: UV/VIS
Natural components are widely used as food and medical products and have long experience of taking it. In this study, it was aimed to develop novel quantitative method of various components in natural products by liquid chromatography (LC) with molar absorption coefficient ratio and high-speed countercurrent chromatography (HSCCC) for safety assessment. In order to evaluate main compounds in products, high-pure references such as standards are needed for LC evaluation. However, it is difficult to obtain these pure standards in common reagent’s company. Thus, we used HSCCC isolation and LC with molar absorption coefficient ratio for accurate quantitative analysis of main components in natural products without referenced standards. HSCCC is that liquid is used of both stationary phase and mobile phase, and large amounts of samples can be injected without irreversible adsorption of the column and loss of the sample. Recovery rate is theoretically 100%. Actually, main components such as sesamin, sesamolin and anthomonasins were isolated from sesame seed oil unsaponified matter and monascus yellow using HSCCC. Then, the molar absorption coefficient ratio was analyzed using HSCCC isolated components for LC evaluation. We also tried developing a quantitative method without standard products.
Powdery mildew disease is a fungal infection which negatively affects crop growth. There is evidence that a novel bio-signaling molecule is present as a result of a gene mutation in A. thaliana that confers the increase in resistance to the disease. Additionally, the same compound has an effect on ethylene signaling. However, the identity of the molecule remains elusive. By using metabolite standards as models for the molecule, a method for isolation and identification is underway. Plant extracts, which contain the metabolite are prepared using C18 solid phase extraction, which is followed by weak anion exchange SPE. A bioassay is then used to confirm if the compound is present in the isolated fractions. The results of the bioassay have led to the hypothesis that the molecule is a highly polar, anionic compound. Further identification of the molecule will include the use of liquid chromatography fraction collection and high resolution NMR spectroscopy. To assist with the isolation and identification, a variety of anionic and neutral metabolite standards have been used to explore hydrophilic interaction liquid chromatography (HILIC). A successful method has been developed which allows for the separation of glucose, sucrose, glutamate, and galacturonic acid from various matrices in a HPLC system. Changing the variables in the method provides insight regarding polar carbohydrate separation. Isolating and identifying the metabolite would provide information regarding the biological mechanism to the resistance of the fungal disease and ethylene sensitivity, which would have a broad impact on the larger agricultural community.
Abstract Text

The identification of bodily fluid (BF) stains at a crime scene is a necessary part of evidence evaluation, but currently has many inherent complexities. This research proves that Raman spectroscopy is a more viable method for testing BF stains than other methods currently in use, specifically for it not being susceptible to false positive (FP) assignments. Currently, different confirmatory tests need to be used for each of the five most common BFs (blood, saliva, semen, sweat, and vaginal fluid). These tests also do not serve the purpose of identifying unknown BFs. Raman spectroscopy is advantageous due to its chemical selectivity, providing unique spectra for all substances analyzed and the ability to correctly identify all types of BFs. By building statistical models, and comparing spectra of BFs to potential false positive substances, all BF stains can be correctly identified with one test.

This study specifically analyzed twenty-four substances that may be misclassified as blood due to their appearance or if known to provide a FP result with currently used tests. Through principal component analysis (PCA), these substances were differentiated from the five aforementioned BFs. The spectra of the twenty-four substances were also loaded into a recently developed support vector machines discriminant analysis (SVMDA) classification model used for BF differentiation. All substances were identified as not peripheral blood, but some of the substances were predicted as one of the other four BFs. A technique known as random forests (RF) was then utilized to build a new classification method to overcome this limitation. The RF model was tested with a validation dataset of BFs and spectra from the twenty-four substances, and complete separation was achieved based on a classification probability threshold of 70%. This method can also be used in the future to test other potential FP substances and confirm that they are not one of the common BFs discovered at crime scenes.

Keywords: Forensic Chemistry, Raman Spectroscopy, Statistical Data Analysis

Application Code: Homeland Security/Forensics

Methodology Code: Molecular Spectroscopy
Microfluidic paper-based analytical devices (micro-PADs) are a growing class of low-cost chemo/biosensing technologies designed for point of care applications. Calcium is an important cation in numerous fields, from environmental monitoring to biomedicine. The goal of this project is to develop a paper-based microfluidic sensor for calcium using immobilized gold nanoparticles. The device is fabricated utilizing a solid ink printer that creates hydrophobic barriers and hydrophilic channels. The fluid is applied to the device and through capillary action, the fluid flows toward the detection region which contains the immobilized gold nanoparticles. We use immobilized functionalized gold nanoparticles as the sensing method for calcium. These nanoparticles are “tunable”, enabling detection of calcium at different concentrations in a sample. We consider the effects of fluid flow and microfluidic device design on the immobilization of the gold nanoparticles and adequate delivery of sample to the reaction region. We also developed a method for analyzing the results to establish a foundation of moving the technique from a qualitative to a semi-quantitative approach. To our knowledge, this is the first attempt to detect calcium on paper using immobilized gold nanoparticles. This proof-of-principle work establishes the foundation for a rapidly deployable microfluidic platform for assessing calcium in resource-limited environments in a point-of-care testing device. Future development on the approach could lead to new clinical applications in the future as well.

This research was supported in part by the Louisiana Board of Regents Support Fund contract number LEQSF(2015-18)-LaSPACE through a subcontract with Louisiana Tech University.
Parkinson’s disease (PD) is a neurodegenerative disease characterized by the slow degeneration of dopaminergic neurons found in a region of the midbrain called the substantia nigra. Dopamine (DA) plays a key role in regulating motor function. Thus, the destruction of these neurons and the consequential decrease in DA concentrations in the striatum leads to the deterioration of motor control. The drug Levodopa has been used to treat PD by helping to increase the concentration of DA in the brain. This drug has been proven to alleviate the motor symptoms of PD; however, after a short period of time, dyskinetic symptoms can develop. Treatment with Citalopram, a serotonin reuptake inhibitor, before administration of Levodopa has proven to attenuate the dyskinesia side-effects. Furthermore, it is thought that oxidative stress is a principal contributor to the destruction of dopaminergic neurons, and possibly to the development of dyskinesias, in PD and its treatment. To date, oxidative stress has been difficult to measure due to the high reactivity of oxygen radicals; however, hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}) can serve as an indicator of the presence of oxidative stress. This experiment uses fast-scan cyclic voltammetry coupled with carbon-fiber microelectrodes to simultaneously monitor real-time fluctuations of DA and H\textsubscript{2}O\textsubscript{2} in the dorsal striatum. These neurochemical dynamics can be time-locked to dyskinetic episodes. Overall, these studies will aid in our understanding of how oxidative stress modulates nigrostriatal DA signaling, as well as the behavioral consequences of this interaction. The results will inform improved therapeutic strategies for the treatment of PD.

Keywords: Electrochemistry, Microelectrode, Neurochemistry, Voltammetry
Application Code: Neurochemistry
Methodology Code: Electrochemistry
Manganese dioxide is a safe and low-cost alternative material for supercapacitor and battery applications. However, its use is currently limited by its poor cyclability and its low ionic and electronic conductivities. To overcome these limitations, many strategies have been centered on the design of nanoparticle to reduce the diffusion distances for the transport of insertion cations. We have employed polymer membranes containing gold nanotubes to synthesize and characterize manganese dioxide nanoparticles. A voltage (2-4 V) was applied across the membrane to generate redox reactions, one cathodic and one anodic, at each end of the gold nanotubes. The anodic reaction was chosen to form MnO$_2$ caps on the nanotubes. The deposition rate and chemical composition of these caps were determined using microscopic, spectroscopic and electrochemical techniques. The ionic conductivity, capacity and permselectivity of these MnO$_2$ particles were also studied before and after discharging in lithium perchlorate. Through this poster presentation, we will provide a new insight to address the performance of this material for applications in batteries and supercapacitors.

**Keywords:** Electrochemistry, Electrodes, Fuels\Energy\Petrochemical, Membrane

**Application Code:** Nanotechnology

**Methodology Code:** Electrochemistry
Conventional drug and vaccine delivery systems are limited in terms of targeted and effective delivery as they distribute the drug evenly and get degraded rapidly. Microneedles (MNs) mediated drug delivery and this system has been developed to self-administer the medications into human’s skin across the Stratum Corneum in a painless and patient-friendly manner alternative to the hypodermic needles. Unlike other MNs, dissolving microneedles (DMNs) do not create any sharp bio-hazardous waste after its use. Current DMNs fabrication methods including photolithography are time-consuming and complicated. In addition, harsh conditions are required for the loading of biological drugs and problems of standardizations also limit their applications. Herein, we report a novel, rapid, and efficient fabrication method of dissolvable microneedles for drug delivery and analysis. A pre-designed template was used to create a polydimethylsiloxane mold by a single step laser engraving technique using a laser cutter. The parameters including, speed and power required for the laser cutter to obtain the desired PDMS mold were optimized. The mold was then placed to mold microneedles in a solution of polyvinylpyrrolidone (PVP) and polyethylene glycol (PEG). The DMN patch was then characterized using Scanning Electron Microscopy (SEM) to verify the shape and height of the DMNs. The DMN will be further tested for in ex-vivo drug delivery and analysis using pigskin. The purposed method of DMN fabrication is easy, low-cost, rapid, and efficient as compared to conventional method of fabrication.

Financial support from NIH, NSF-PREM, MCA Foundation, UT STARS Award, MRAP, IDR, and URI Award from UTEP is gratefully acknowledged.

**Keywords:** Biomedical, Dissolution, Drugs, Microscopy

**Application Code:** Biomedical

**Methodology Code:** Microfluidics/Lab-on-a-Chip
Due to the small channel size, microfluidic devices are particularly susceptible to fouling by cells or biomolecules. Supported lipid bilayers are a convenient solution to this problem because they spontaneously form on the inside of hydrophilic channels and serve to reduce adhesion of sample components. Our research goal is to understand how the composition of a supported lipid bilayer affects its performance in a microfluidic device for single-cell analysis of the social amoeba [i] Dictyostelium discoideum. [i] To study the effect of net bilayer charge on adhesion of [i] Dictyostelium discoideum [i] cells, the mole percent of a negative phospholipid, phosphatidylglycerol, was increased. [i] Dictyostelium [i] cells have a slight negative charge, therefore when the negative character of the bilayer was increased, cell adhesion was expected to decrease. The bilayer composition varied from 0 to 30% phosphatidylglycerol (PG) with the remainder consisting of zwitterionic phosphatidylcholine. Cell adhesion was measured by allowing cells to adhere to the channels for 15 minutes, then counting the cells before and after rinsing the channels. Although it was originally suspected that increased negative character would reduce cell adhesion, preliminary data suggests that the opposite may be true. Due to this unexpected trend, the effect of a net positively charged bilayer was also studied. By better understanding how lipid composition influences failure by fouling, microfluidic devices can be optimized for single cell analysis.

Keywords: Bioanalytical, Lab-on-a-Chip/Microfluidics, Lipids
Application Code: Bioanalytical
Methodology Code: Microfluidics/Lab-on-a-Chip
Human Serum Albumin, HSA, a major transport protein represents 55-60% of the total plasma protein. It serves as depot and career for many endogenous [fatty acids, bilirubin, etc.] and exogenous ligands [drugs, metabolites, nutrients, etc.]. Studies on ligand/drug–protein binding are vial from both theoretical and physiological perspective as they allow better understanding of the processes underlying drug disposition and elimination and the effect of several pathological states or co-administered drugs on drug delivery and efficacy. Interestingly, unconjugated bilirubin is transported mainly bound to HSA. Lone tryptophanyl-214 of HSA lies in domain II allows utilizing the fluorescence spectroscopy very effectively by quenching due to binding to various ligands. We have used a defatted and double chemically modified [~18% buried lysine core] HSA to explore molecular basis of ligand binding and bilirubin displacement. We have used conventional data analysis for binding constant computation as well as alternative approach. Our results with analgesic, salicylic acid indicated with matching binding pattern with negative enthalpy and Ho, free energy change, compared to bilirubin suggesting that it share bilirubin binding pocket. Additionally previous studies with fatty acid analogue, DAUDA using spectroscopy and crystallography had revealed as contradictory outcome, i.e., one in closer proximity to bilirubin and other to classical fatty acid binding site. Interestingly, our DAUDA study using fluorescence enhancement to probe bound bilirubin displacement indicate distinct motif in bilirubin binding proximity. Significant binding loss with modified HSA, strengthen our results. Finally, the displacement of bilirubin from ligand-albumin complex allowed us to predict the competitive model of displacement due to binding/sharing binding pocket will be presented to outline the molecular basis of displacement.

Keywords: Fluorescence, Protein
Application Code: Biomedical
Methodology Code: Fluorescence/Luminescence
Polyethylenimine (PEI) is a cationic polyelectrolyte that is widely synthesized in both linear (L) and branched (B) forms and with varying molecular weights. While PEI is well-known in gene therapy for DNA transfection, various research has shown that this cationic polyelectrolyte also lead to various levels of antimicrobial activity. It was determined that differences in membrane polarization are most likely due to electrostatic interactions between PEI and the bacteria. The molecular weight (MW) of PEI is also an important factor to investigate its antimicrobial properties. Research has shown that lower MW of BPEIs at 600 Da have been found to be less cytotoxic to human cells compared to other BPEIs with higher MW (i.e. 10,000 Da). This study aims to investigate the correlation between the electrostatic and structural differences in BPEI in at least 4 samples of differing molecular weights. The molecular weights of the BPEI samples were determined by the methods used by their manufacturing company, and quantitative charge analyses of the particles were accomplished by using a particle charge detector (PCD). The PCD uses the streaming current principle by finding the point of zero charge and followed by a titration. The charge density measurement will be identified for each BPEI particle. While the majority of the study has been on varying MWs of BPEI, experiments were performed to replicate a gene transfection protocol prior to the binding of PEI and DNA to observe the polyelectrolytic material. Further correlation statistical analyses will be conducted to investigate the relationship of charge density and MW in the BPEI particles tested. If successful, this study will elucidate the mechanism of PEI’s interactions with biological structures, thereby explaining the particles’ observed selectivity. Furthermore, this experiment will show that the particle charge detector is a valuable tool to measure the charge density of polyelectrolytic material.

Keywords: Bioanalytical, Materials Characterization, Particle Size and Distribution, Polymers & Plastics
Application Code: Bioanalytical
Methodology Code: New Method
Nanoaperture optical trapping is a technique that uses nanoscale aperture antennas fabricated into a 100 nm thick gold film to trap and subsequently study single proteins for biochemistry and biophysics applications. Current methods for constructing these nanohole antennas require direct-writing techniques, such as electron-beam lithography or focused ion beam (FIB) milling, for each sample. These techniques are slow, expensive, and introduce manufacturing variances in the nanohole structures. Template stripping methods provide an inexpensive, scalable, and faster solution to direct-write techniques. Our goal is to develop a quick and reliable method for fabricating double nanohole antennas, which will be used to trap single proteins more consistently and with more trapping strength. As a result, these traps will trap the protein for longer using less optical power. To minimize the necessity for using direct-write techniques, we plan to fabricate these nanoscale traps by using the FIB to produce a master in silicon, then depositing gold directly onto the silicon and lifting off the gold film using a UV curable epoxy. After FIB the patterns in the silicon will be etched down to a micron to allow the evaporated gold to pass through the aperture and be lifted off without any surface peeling effects. The traps consist of a roughly 100 nm thick gold film, which we will peel off the master onto a UV epoxy coated glass slide. With nanohole depths in the master of around a micron, it can be reused about 5 times before needing to clean it. The efficiency of this method far outweighs that of direct-writing nanohole apertures for each sample, as the cleaning process of the master can easily and inexpensively be done with a liquid gold etchant. This template stripping based nanofabrication technique would greatly reduce the time and cost spent on fabricating each sample, and would also increase the fabrication consistency of the nanohole traps.

Keywords: Lab-on-a-Chip/Microfluidics, Laser, Nanotechnology, Protein
Application Code: Nanotechnology
Methodology Code: Microfluidics/Lab-on-a-Chip
### Abstract Text

Organo-lead halide perovskites have emerged as a potential absorbance material for implementation in photovoltaic devices. Recent techniques have differentiated lead perovskites that contain a different anion halide group. Both CH[sub]3[/sub]NH[sub]3[/sub]PbI[sub]3[/sub] and CH[sub]3[/sub]NH[sub]3[/sub]PbCl[sub]3[/sub] perovskites were formed using two separate methods of film formation in order to compare ligand interactions with the crystalline surface. UV-visible spectroscopy was implemented in order to confirm the presence of a bandgap for each of the perovskite samples. In addition, SEM and XRD was measured in order to characterize the perovskite crystal structure and investigate the presence of lead halides or methylammonium iodide (MAI) on the surface. Overall, photoluminescence lifetimes increased with increasing ligand Lewis basicity for the CH[sub]3[/sub]NH[sub]3[/sub]PbCl[sub]3[/sub] perovskites. However, minimum lifetime improvement was observed for CH[sub]3[/sub]NH[sub]3[/sub]PbI[sub]3[/sub] perovskites with increasing ligand Lewis basicity due to predicted ligand interaction with the halide ion.

**Keywords:** Fuels\Energy\Petrochemical, Luminescence, Spectroscopy, UV-VIS Absorbance/Luminescence

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

**Methodology Code:** Fluorescence/Luminescence
With the rising cancer survival rates in the past few decades, interest is turning from simply keeping patients alive to treating patients in a way that will ensure them a good quality of life afterwards. One of the biggest quality of life issues that cancer survivors have to deal with is chemobrain, which can last many years after treatment. This condition is characterized by symptoms such as impaired language ability, difficulty concentrating, a shorter attention span, and memory issues, all of which can dramatically affect a person’s quality of life after treatment is finished. Chemobrain has been linked to low brain dopamine levels, but the mechanism behind the condition is still unknown, and as such no treatment or cure has been created. This study used fast-scan cyclic voltammetry to measure dopamine levels from zebrafish whole brains after a two-week treatment regime with either carboplatin or 5-flourouracil, or with the control, saline. The fish were allowed different times to recover from the treatment before their measurements were taken in order to determine if the brain dopamine levels recovered to the control levels.

**Keywords:** Bioanalytical, Electrochemistry, Neurochemistry, Voltammetry

**Application Code:** Bioanalytical

**Methodology Code:** Electrochemistry
Abstract Text

[alpha]-Synuclein ([alpha]S) is a protein found in neural cells and are often a large component of Lewy bodies. The presence of Lewy bodies is often a sign of Parkinson’s Disease. The protein is known to interact and bind with lipid membranes in neuronal cells. The interaction with the membranes is thought to help aid in regulation of transmitter releases. While the membrane interaction of [alpha]S can be observed, its function is still under investigation.

Previous works have been done to show that membrane binding changes the structure of [alpha]S from disordered to an amphipathic helix, allowing curvature sensing capabilities. These techniques only look at whether or not the [alpha]S binds to the membrane. Our work differentiates itself by looking at the binding of [alpha]S at a residue level, rather than a macroscopic view of the whole protein.

One method of observing the structure during binding of [alpha]S is by using electron spin resonance (ESR). ESR requires a spin-labeled protein. The spin label can only be attached after going through site directed mutagenesis to put a cysteine residue in the protein.

The objectives of this project were to express, purify, and spin label mutated [alpha]S. ESR spectroscopy was performed on the samples in the presence of vesicles to probe membrane binding. To this date, data has been collected on several [alpha]S mutants, including the A30C mutant generated at Westminster College. Though there were some spin-labeling issues, ESR spectra were collected on Q24C and S9C showing little (or no) binding at this level. Currently, protocols are being optimized to improve spin labeling and membrane binding.
Trace metal concentrations in hair are biomarkers for human health, nutrition, and environmental exposure. Deficiency or excess of certain metals impacts biochemical and metabolic reactions within the body and can be correlated to diseases. Hair analysis is non-invasive, and the samples have higher metal concentrations compared to other biological samples such as blood or urine. A survey of the literature showed there are many different trends in hair samples that are significant. Forty hair samples were collected along with surveys of each individual that donated hair in order to search for trends within the Westminster College student population. Hair samples were digested using nitric acid and analyzed using inductively coupled plasma optical emission spectroscopy. Correlations among metals and trends depending on demographics, lifestyle, health, and nutrition were analyzed using statistics. Examples of preliminary trends are differences in Na and K concentrations with smoking status and differences in Na, As, and K with age.

Keywords: Bioanalytical, Biological Samples, ICP, Statistical Data Analysis
Application Code: Bioanalytical
Methodology Code: Atomic Spectroscopy/Elemental Analysis
Cultivating Chemistry: Analysis of THC and CBD in Hemp Products as an Alternative to Cannabis Testing in the Undergraduate Lab Setting

The chemical study of the Cannabis sativa plant is continuing to gain important prevalence in today’s society. To assist undergraduate chemists in preparation for the vast career possibilities that the cannabis field will offer, an undergraduate lab pertaining to the study of important cannabis compounds was developed. Through the use of reversed phase high-performance liquid chromatography (HPLC), a central technique for the analysis of cannabis and other illicit substances, the analysis of tetrahydrocannabinol (THC) and cannabidiol (CBD) can be performed simply and effectively. Due to the classification of cannabis as a controlled substance, the analysis was performed on products of the hemp plant, a variety of Cannabis sativa grown for its industrial fibers instead of its medicinal and psychoactive properties. Three different hemp oil products were analyzed via HPLC, and results demonstrate clear resolution of quantifiable THC and CBD peaks. A lab incorporating the analyses of these compounds could see use in analytical chemistry curricula, along with potential applications in other subdisciplines, such as organic chemistry and biochemistry. Exposing undergraduate chemists to this type of analysis would assist them in gaining experiences and knowledge that could potentially lead to a career in the growing field of cannabis chemistry.

Keywords: Education, HPLC, Natural Products
Application Code: Other
Methodology Code: Education/Teaching

Exposition Floor, Aisles 2000-2700
Wednesday, February 28, 2018 - Aft
Abstract Text

Rare earth elements are often used in common electronics and engineering in the form of rare earth magnets, such as samarium cobalt magnets, due to their ability to stay magnetic at high temperatures and their strong magnetic properties. Demand for rare earth magnets continues to increase; however, traditional extractions of rare earth elements are expensive and generate a lot of waste. Rare earth elements are also traditionally separated by the use of concentrated mineral acids and harsh organic solvents, which are dangerous and costly. Ionic liquids have been used to separate samarium from cobalt recently, but this process caused problems in the extraction due to high viscosity solvents. Hydrophobic deep eutectic solvents (DES) have emerged as an alternative extraction method from ionic liquids. These solvents require less purification steps and have been used in separations, catalysis, organic synthesis, and other processes.

In this research, two hydrophobic DESs were examined to determine the extraction mechanism for the extraction of cobalt from samarium cobalt magnets. These DESs contained tetraoctylammonium bromide and a carboxylic acid, oleic acid and lauric acid were both used. Each DES was mixed with a samarium cobalt magnet digested in hydrochloric acid. Then samarium and cobalt concentrations in the aqueous extraction layer were quantified using ICP-OES in our previous research. It was theorized that the cobalt was extracted in a bromide chloride exchange mechanism. HPLC was performed to determine if a bromide chloride exchange mechanism could be observed in this reaction’s saturation of chloride ions. Cobalt was successfully extracted in the form of cobalt tetrachloride as show by our professionally verified HPLC results.

Keywords: Environmental, Extraction, HPLC, Method Development
Application Code: Environmental
Methodology Code: Separation Sciences
Undergraduate Poster Session

Inexpensive 3D Printable Raman Spectrometer for Undergraduate Learning Experiences

The ability to perform spectroscopy and interpret spectra are invaluable tools for chemical identification, quality control analysis, or chemical research. For this reason, the importance of spectroscopy is emphasized throughout the course of undergraduate study for chemistry majors. Raman spectroscopy is a form of vibrational spectroscopy, similar to infrared spectroscopy but with different selection rules that lead to complimentary information. However, Raman spectroscopy is not taught in many Chemistry departments due to instrumentation costs and faculty inexperience with the technique. The lack of analytical capability for Raman spectroscopy in undergraduate institutions prevents hands on exposure. Early experience with Raman spectroscopy could lead students to consider Raman as a technique to solve problems in future research or career projects. While we cannot directly tackle instructor inexperience with the technique, this study explores the design and production of a low-cost Raman spectrometer for undergraduate study. The goals of the project are to design an instrument with a 532 nm laser, a modifiable 90° or 180° sampling geometry, and XXX wavenumber resolution for a total price tag around $1000. While we are using commercial optical components (mirrors, diffraction grating), all of the optical mounts and housing are being designed and 3D printed in house. When complete, all plans, 3D printing files, and developed software will be made freely available. The current state of development and comparison with similar instruments available online will be presented.

Keywords: Instrumentation, Raman Spectroscopy, Teaching/Education, Vibrational Spectroscopy

Application Code: Other

Methodology Code: Education/Teaching
Undergraduate Poster Session

**Abstract Title**
The Effect of UV Light Irradiation on α-Crystallin Chaperone Activity

**Primary Author**
Tristan Toca
Westminster College

**Co-Author(s)**
Erin Wilson

**Abstract Text**

α-Crystallin is a lens chaperone protein that works to prevent cataracts from forming by refolding denatured proteins before they aggregate. When α-crystallin begins to lose its chaperone activity, cataracts begin to form. It has been known that α-crystallin loses its activity after extended exposure to ultraviolet light radiation. It was recently discovered, however, that when α-crystallin is exposed to UV light for short amounts of time, the activity increases. Since this is not something that has been extensively studied, it is unknown how the conformation of α-crystallin changes when exposed to UV light for brief periods of time. To better understand how the conformation changes, a light scattering analysis was used to determine the amount of UV exposure that led to the greatest α-crystallin chaperone activity. Fluorescence analysis was also used to determine how the conformation of α-crystallin changes with increasing amounts of UV light exposure. Finally, a series of gels, both SDS and native PAGE, were used to determine both the strength of covalent and hydrophobic interactions between subunits in an oligomer of α-crystallin and the average number of subunits in an oligomer of α-crystallin with both short and long UV light exposures. The light scattering analysis showed that α-crystallin was most active after approximately 20 minutes of UV light exposure. The fluorescence indicated that tryptophan was being destroyed by the UV light and that the oligomer was burying the fluorescent amino acids in more hydrophobic locations. The gels are still currently being run but what is known so far is that with increasing UV exposure, there is a high molecular weight band that does not enter the SDS gels with and without a reducing agent. In light of the fluorescence results, this indicates that the more hydrophobic the oligomers become, the stronger their bonds become, which inevitably hinder the activity as a whole.

**Keywords:** Biomedical, Fluorescence, Molecular Spectroscopy, UV-VIS Absorbance/Luminescence

**Application Code:** Biomedical

**Methodology Code:** Molecular Spectroscopy
Cytochrome c (cyt c) is a protein that transports electrons from cyt c reductase to cyt c oxidase found in the mitochondria of cells. The movement of electrons in the electron transport chain can be mimicked in vitro using cyt c adsorbed to self-assembled monolayers (SAMs) covalently bonded to evaporated gold. Many papers have been published investigating the interaction of cyt c with carboxylic acid terminated alkanethiol films on gold. Since cyt c interacts with protein in the electron transport chain, a better interaction surface to mimic the natural system would be peptide SAMs. Electrodes were constructed to evaluate cyt c thermodynamics and kinetics by assembling the peptide on evaporated gold/glass. Cyt c adsorbs to the peptide SAM because the terminal end of the SAM exposed to solution has a net negative charge and attracts the positively charged lysine groups in the cyt c.

The purpose of this research is to become more familiar with the interaction between peptide SAMs and cyt c to investigate potential enhancement by the peptide protein interaction. The peptide SAM studied was comprised of glutamic acid, alanine, and cysteine. The data obtained will be compared to past research using alkanethiol SAMs. The current full monolayer cyt c/peptide data shows the cyt c on the peptide SAMs has a lower electron transport rate than cyt c on the alkanethiol SAMs. The peptide SAMs being tested also showed a higher capacitance for the background cyclic voltammograms.

The peptide SAMs were synthesized using two different procedures (traditional solid phase peptide synthesis and microwave peptide synthesis) and compared. When comparing the two differently synthesized peptide SAMs, there was no significant difference in electron transport rate for cyt c. In addition to full peptide monolayer SAMs, mixed monolayers of peptide and shorter chain alkanethiols have been explored. Results will be presented for all of the cyt c/peptide monolayers investigated.

**Abstract Text**

**Keywords:** Electrochemistry, Peptides, Surface Analysis, Voltammetry

**Application Code:** Bioanalytical

**Methodology Code:** Electrochemistry
Eye cancer is an extremely rare cancer, though, ocular melanoma is the most common type of eye cancer in adults. Between 2500 and 3000 adults are diagnosed with ocular melanoma every year in the United States. Ocular melanoma develops in pigmented cells of the choroid, iris or ciliary body. Ocular cancer treatment depends on the age of the patient, location of the cancer, patient preferences, and his/her overall health. Surgical options are removal of part of the iris, the iris and ciliary body, choroidal tumor, or the entire eye. Radiation therapy can also be used as a treatment though laser therapy is preferred because there are fewer side-effects. Drug therapy has also been known to be successful in the treatment of ocular melanoma. Cyclophosphamide, a well-known pharmacological compound with cytotoxic properties, is often used for treatment. However, preceding research has identified another potentially useful drug, Quinoline-3-carboxamide-Linomide (LS-2616). Previous research projects have investigated the effects of LS-2616 at different stages of tumor formation, and compared the effects of LS-2616 to those of cyclophosphamide. The effects of LS-2616 on mice, have been quite comparable to the success with those of cyclophosphamide. These successes suggest a potential role for LS-2616 in the treatment of ocular cancer.

The goal of the project is to propose and carry out a green, organic multistep synthesis of LS-2616. In each step of the synthesis, the reaction conditions will be optimized with reagent availability, time, safety, and green chemistry considerations in mind. Most reaction conditions in the scheme are from known procedures and use reactions discussed in organic chemistry classes. As the literature reaction conditions were proposed for compounds that are similar to our substrates, it is necessary to optimize reaction conditions for each individual reaction. Currently, the reaction conditions for the methylation of the amine are being optimized.

Keywords: Chemical, Drugs, FTIR, NMR
Application Code: Pharmaceutical
Methodology Code: Vibrational Spectroscopy
Mitochondrial Transcription Factor A (TFAM) is a protein that controls the copy number of mitochondrial DNA. It has been speculated that TFAM has effects on the heredity of diabetes and other disease and X-linked disorders. Understanding the way that TFAM functions could be an important stepping stone in medical treatment. Therefore, finding the best way to purify TFAM from cell matter is the first step in observing the effects that it can have on health. To purify TFAM, specialized E.coli cells were grown with a plasmid that codes for His-SUMO-TFAM. We adopted a recently published purification method that utilizes different and more drastic changes in the concentrations of imidazole in wash buffers and compared the purity of TFAM extracted by this method to our previously used purification procedure. In the old procedure, the column was initially washed with both 5 mM imidazole and the TFAM was eluted with 50 mM imidazole. In the new procedure, the column was washed with both 5mM and 10 mM imidazole and the TFAM is eluted with 150 mM imidazole. The protein was visualized using SDS polyacrylamide gels. In comparison to results obtained from the previous method, the gels showed a reduced amount of contamination in the collected product. Using the new procedure utilizing batch binding, higher centrifugation speeds, and different imidazole concentrations, more bacterial protein and DNA were washed away, resulting in more pure TFAM in the final collected product.

Keywords: Biological Samples, Chromatography, Electrophoresis, Protein
Application Code: Other
Methodology Code: Capillary Electrophoresis
The Comparison of α-Crystallin and Caffeine in the Prevention of Cataracts in Bovine Lenses

For many individuals, cataracts can cause severe vision loss, which leads to unforeseen expenses and decreased quality of life. Cataracts form through the aggregation of misfolded peptides, which are the result of oxidation reactions. Naturally, α-crystallin proteins act as a chaperone to prevent these reactions. However, the amount of α-crystallin proteins present in the body is limited. Likewise, antioxidants such as caffeine halt oxidation reactions, and potentially preventing cataract formation. Therefore, the goal of this study was to build upon previous studies focused on determining alternate pharmacological methods of preventing the development of cataracts.

In order to determine possible compounds which could inhibit the development of cataracts in, the preventative effectiveness of α-crystallin and caffeine was compared. This was achieved by obtaining bovine lenses, which were treated with solutions of either caffeine or α-crystallin, as well as a fixed amount of sodium selenite, which was added with the goal of expediting cataract formation via biochemical redox reactions. After 20 days of the dual exposure of selenite and caffeine, the development of cataracts was analyzed qualitatively through observations, and quantitatively through measuring the concentration of calcium in digested lenses via a Thermo S Series Flame Atomic Absorption Spectrometer. Calcium concentrations were determined by analyzing each supernatant via spectrometry, since previous research suggested that higher concentrations of calcium indicate cataract formation. Qualitative evidence showing the development of cataracts suggested that cataract formation was slowed by the use of a caffeine solution treatment. However, statistical analysis of the measured calcium concentrations in the lenses showed that no conclusive evidence about the formation of cataracts was obtained. Further trials are being completed in order to verify the results of the study.

Keywords: Atomic Absorption, Pharmaceutical, Protein, Spectrometer
Application Code: Biomedical
Methodology Code: Atomic Spectroscopy/Elemental Analysis
Dissecting the inner workings of a cell requires imaging methods with molecular specificity, molecular-scale resolution, and dynamic imaging capability such that molecular interactions inside the cell can be directly visualized. However, the diffraction-limited resolution of light microscopy is substantially larger than molecular length scales in cells, making many sub-cellular structures difficult to resolve. Another major challenge in imaging is the relatively low throughput in terms of the number of molecular species that can be simultaneously imaged, and genomic-scale throughput is desired for investigating many systems level questions. In this talk, I will describe two imaging methods that overcome these challenges and their biological applications. I will first describe stochastic optical reconstruction microscopy (STORM), a super-resolution imaging method that circumvents the diffraction limit. This approach has allowed multicolor and three-dimensional imaging of living cells with nanometer-scale resolution. I will present both technological advances and biological applications of STORM, with focus on some recent biological discoveries of cellular structures enabled by STORM. I will also describe our recently developed single-cell transcriptome imaging method, multiplexed error-robust fluorescence in situ hybridization (MERFISH), which allows thousands of RNA species to be simultaneously imaged in individual cells. This approach enables single-cell transcriptomic analysis in the native context of tissues, facilitating the delineation of gene regulatory networks, the mapping of RNA distributions inside cells, and the mapping of distinct cell types in complex tissues.

Keywords: Imaging, Microscopy, Nucleic Acids, Single Molecule
Application Code: Biomedical
Methodology Code: Microscopy
Imaging has been an essential tool to analyze the dynamic properties of RNA. Newly developed new technologies in optical microscopy and novel methods for RNA tagging have allowed us to detect and track individual mRNA molecules in single living cells, yielding insights that could not have been obtained through any ensemble measurement. These approaches employ a plethora of imaging techniques, ranging from multiphoton microscopy, high-speed real-time widefield microscopy, single molecule tracking, super-registration microscopy and fluorescence fluctuation analysis. The kinetics of the key elements of RNA metabolism: initiation, elongation, termination, export, cytoplasmic trafficking, localization, translation and decay are now tractable at the single molecule level in real time in living cells. For instance it was possible to measure the initiation frequency and elongation rates of single mRNA transcription or translation, or the probability and time to be transported through a nuclear pore. We have been dedicated most recently to developing and implementing these technologies to further the understanding of dynamics of mRNA regulation in living tissues, particularly neural tissues of a transgenic mouse where the mRNAs synthesized from targeted genes contain stem-loops from a phage bound by a fluorescent capsid. By observing RNA in its native environment, we may find new regulatory mechanisms. Supported by funding from NIH.
DNA exists as single molecules in individual cells. Consequently, genomic variations such as copy-number variations (CNVs) and single nucleotide variations (SNVs) in a single-cell occur in a stochastic way, necessitating single-cell and single-molecule measurements to be identified. However, existing single-cell whole genome amplification (WGA) methods are limited by low accuracy of CNV and SNV detection. We have developed transposase-based methods for single-cell WGA, which have superseded previous methods. With the improved genome coverage of our new WGA method, we have also developed a high-resolution single-cell chromatin conformation capture method, which allows for the first 3D genome map of a human diploid cell.

Gene expression is also stochastic due to the fact that the DNA exists as single-molecules in individual cells. The correlations among different mRNAs in a single-cell are masked within the stochastic gene expression noise. We have developed a method for single-cell transcriptome with improved detection efficiency and accuracy, revealing intrinsic correlations among all detected mRNAs in a single-cell. For a particular human cell type, we uncovered ~120 transcriptionally correlated modules (TCMs) from the gene expression data of ~700 individual cells under a steady state condition. We found that the TCMs are cell type dependent.

Keywords: Biological Samples, Biomedical, Genomics, Single Molecule
Application Code: Genomics, Proteomics and Other ‘Omics
Methodology Code: Chemical Methods
### Abstract Text

In order to elucidate how a large number of protein and nucleic acid molecules orchestrate cellular processes in a small volume, we take a microscopy approach that systematically maps their spatial distribution, activity profile and temporal dynamics. We have developed the CRISPR imaging method to visualize endogenous genomic elements in living cells. We have also developed a scalable method to edit human genes for split-fluorescent-protein tagging, enabling both microscopy and biochemical analysis of endogenous proteins in the native cellular context at the genomic scale.

**Keywords:** Microscopy, Nucleic Acids, Protein

**Application Code:** Biomedical

**Methodology Code:** Microscopy
Surface enhanced Raman spectra of whole human blood and blood components excited at 785 nm on both Au and Ag substrates have been observed. These spectra are found to be highly dependent on sample preparation protocols. SERS spectra of fresh whole blood placed on a Au nanostructured substrate (785 nm excitation) are dominated by a few molecular components of the blood plasma; uric acid and thiocyanate are readily identified. This is in contrast to the normal Raman spectra which are dominated by oxy-hemoglobin (oxyHb) at this same excitation wavelength. As blood ages, the SERS spectrum of whole blood exhibits a transformation from uric acid to hypoxanthine. Aside from this observation demonstrating the capacity for SERS to detect the appearance of exogenous metabolites from living blood cells and thus the ability of SERS to monitor in vitro biological processes, SERS provides a convenient measure of blood aging. SERS of red blood cells (RBCs) and oxyHb are compared and contrasted with the corresponding normal Raman spectra of both excited at 785 nm. SERS spectra of RBCs and oxyHb on Au and Ag are observed and significant reproducible differences, attributable to redox activity on the Ag substrates are evident. New bands are strongly enhanced in the SERS spectra of RBCs and Hb, relative to normal 785 nm spectra, and DFT calculation have been carried out to identify these vibrational features. Comparison of Hb, hematin and [beta]-hematin demonstrate how SERS may be used as a rapid malaria diagnostic. Finally we show how SERS can be used to detect and identify trace amounts of blood for forensic applications. Unprecedented levels of sensitivity are achievable with SERS for on-site crime scene investigations.
Blood is an intensely complex mixture of particles and molecules. Nearly all analytical approaches to monitoring components within that mixture require some sort of separations (centrifugation, for example). Our group is developing better separation methods for targets ranging from proteins to ten micron particles, including virtually all biological targets (proteins, organelles, bioparticles, cells, etc.) Using gradient techniques for separations of complex mixtures allows for the effects diffusion and other dispersive factors to be minimized. An especially attractive mix of forces for common biological samples is electrophoresis and dielectrophoresis and I will focus on a technique which sets electrophoretic forces to oppose dielectrophoretic ones within an open channel. In this channel, there are unique capture points for various values of electrophoretic and dielectrophoretic properties of the analytical targets, described by characteristic mobilities ($\mu_{EK}$ and $\mu_{DEP}$, respectively). With this technique, very similar particles and biological materials can be efficiently separated. Under optimal conditions a one micron particle can be separated from one that varies by only one nanometer (one part in a 1000-2000). Other characteristic resolution factors include minimal resolvable $\mu_{DEP}$ at $10^{-26}$ m$^4$/V$^2$s (one part in $10^9$) and minimal resolvable Clausius-Mossotti factor at $10^{-9}$. These are extraordinary figures of merit. We have already shown extremely positive results by separating gentamicin resistant Staphylococcus epidermidis from susceptible strains, reflecting extremely subtle alterations in cellular structure or function. A theoretical framework and resulting predictions will be presented along with key data supporting the models.

Keywords: Bioanalytical, Capillary Electrophoresis, Particle Size and Distribution
Application Code: Bioanalytical
Methodology Code: Separation Sciences
Liquid biopsies are generating great interest within the biomedical community due to the simplicity for securing important biomarkers to manage complex diseases, such as many of the cancer-related diseases using a simple blood draw. Liquid biopsy markers can facilitate the realization of Precision Medicine in terms of discovering new patient-specific therapies and stratifying patients so they can be matched to appropriate chemotherapeutic agents. Liquid biopsy markers consist of CTCs, cfDNA and exosomes. We are developing a suite of novel microfluidic devices that can process whole blood directly and engineered to efficiently search for a variety of disease-associated liquid biopsy markers from divergent populations comprising the tumor microenvironment that can supply complementary clinical information. Each microfluidic device can isolate the target with recovery >90% and sufficient purity (>80%) to enable downstream molecular analysis of the particular biomarker. For CTCs, an array of sinusoidally-shaped microfluidic channels surface decorated with antibodies are used for the affinity enrichment of these cells directly from whole blood. In the case of cfDNA and exosomes, high density arrays of micropillars (5 µm diameter with a 5 µm spacing) are used to enrich these biomarkers from plasma. For exosomes, antibodies similar to those used for CTC enrichment are used to secure only the disease-specific exosomes. In the case of cfDNA, extraction of the plasma-based cfDNA is enabled using an immobilization buffer consisting of PEG and MgCl2. The microfluidic devices are made from thermoplastics via injection molding to allow for mass-production to accommodate clinical implementation. In this presentation, information will be shared on the operational parameters of these devices for the selection of liquid biopsy markers, and the downstream molecular information that can be garnered from the isolated markers.

Keywords: Bioanalytical, Biotechnology
Application Code: Biomedical
Methodology Code: Microfluidics/Lab-on-a-Chip
Advances in Blood-Based Diagnostics Using Raman Scattering and Other Techniques

Direct Biochemical Characterization of Stored Red Blood Cells Using Raman Spectroscopy

Donated red blood cells (RBCs) continue to metabolize via the glycolytic pathway and degrade during storage in blood bags at 4-6°C. Transfusion with older, degraded blood products underlies poorer therapeutic efficacy, and the potential for transfusion-associated toxicity. Current methods used for RBC screening are invasive and time-consuming. Thus, improving diagnostic ability to assess the quality in a given until of stored RBC remains a key goal in transfusion medicine. Resonance Raman spectroscopy is a label-free modality that provides enhanced Raman spectra of molecules associated with electronic transitions in resonance with the excitation wavelength. In this study, we discuss recent efforts in developing a novel strategy - Diffuse Resonance Raman Spectroscopy (DRRS) – to acquire depth-sensitive Raman spectra. Two main aspects of this methodology include i) illumination by a diffuse excitation beam which decreases photon density and penetrates further into the sample, and ii) inclusion of additional CCD detector pixels adjacent to those aligned with the optical path to augment the detection of diffuse photons. Unlike excitation with near-infrared wavelengths, DRRS requires ten-fold less power and acquisition time to get a comparable signal-to-noise ratio. We employed this approach for identification of the spectral features of hemoglobin that characterized the age-dependence of RBCs contained inside blood bag segments underneath a 1 mm polymer layer. Statistical analysis performed on these Raman spectra classified young (6-8 days) and old (35-42 days) stored RBCs with >95% sensitivity and specificity. Based on these results, it is evident that DRRS is a rapid, non-invasive and non-destructive approach to attain subsurface information for RBC-related molecules in resonance with the excitation wavelength, and has the potential for in-vivo applications targeting molecules such as heme proteins and carotenoids.

Keywords: Bioanalytical, Biomedical, Biospectroscopy, Raman Spectroscopy
Application Code: Biomedical
Methodology Code: Vibrational Spectroscopy
Diagnostic tests for tuberculosis (TB) are at the first line of defense in patient care and global infection control. This presentation describes the development and assessment of a heterogeneous immunoassay for TB. The assay is based on surface-enhanced Raman scattering (SERS) for the low-level detection of mannose-capped lipoarabinomannan (ManLAM). ManLAM is a lipoglycan unique to the cell wall of mycobacteria, major virulence factor in the infectious pathology of TB, and has been found in the serum, sputum, and urine of infected patients. The assay integrates gold nanoparticle labels, monoclonal antibodies, and SERS, which, when combined with a simple serum pretreatment procedure, enables the effective measurement of LAM in human serum. Pretreatment disrupts the complexation of ManLAM with proteins and other serum constituents, which sterically hinders ManLAM capture and/or nanoparticle labeling. To test the effectiveness of this approach, a preliminary assessment was carried out using serum from TB-positive patients (culture-confirmed and HIV negative) and healthy controls. The results: (1) demonstrate the potential strengths of this approach as a new diagnostic test for this disease, and (2) provide much needed evidence of the clinical utility of LAM as a TB biomarker. Prospects and challenges to the extension of this approach for use in clinics and point-of-need settings, along with possible applications to other TB markers and body fluids, are briefly examined and discussed.

Keywords: Bioanalytical, Clinical Chemistry, Immunoassay, Surface Enhanced Raman Spectroscopy
Application Code: Biomedical
Methodology Code: Sampling and Sample Preparation
Aptamers that bind cell surface receptors represent an important class of ligands for the development of diagnostics and therapeutics. However, it is difficult to compare the function and specificity of many of these molecules when examining published literature, especially when it comes to binding and targeting live cells. We chemically synthesized 15 aptamers that have been reported to target a range of human cell surface receptors associated with cancers. Using a standardized set of conditions, we utilized flow cytometry to assess each aptamer’s cell binding properties on a panel of 11 different cancer cell lines. For aptamers with a reported cell surface target, we confirmed specificity by correlating aptamer binding to an antibody control and by using siRNA transfection to knock down the reported cognate receptor. Using near-infrared in vivo imaging, we assessed the tumor homing properties of a subset of these molecules on prostate tumors implanted subcutaneously in nude mice to correlate the observed in vitro activity with in vivo activity. Our data demonstrate some surprising differences in the reported specificity for many of these molecules and raise concerns regarding the cell targeting capabilities of some of these molecules. The therapeutic effects and diagnostic capabilities reported for many of these molecules could likely be significantly improved through the use of more robust molecules, with improved specificity for their target.
A full understanding of the molecular basis of diseases depends on the development of molecular probes able to recognize disease targets of interest. Until very recently, such tools have been absent from the clinical practice of medicine. The newest molecular probe, and one that holds most promise, is a new class of designer nucleic acids, termed aptamers, which are single-stranded DNA/RNA able to recognize specific targets, such as single proteins and even small molecules. Recently, we applied a simple, fast and reproducible cell-based aptamer selection strategy called Cell-SELEX which uses whole, intact cells as the target for aptamer selection. This selection process then generates multiple aptamers for the specific recognition of biological cells, but without the need for prior knowledge about the signature of target cell-surface molecules. The selected aptamers have dissociation constants in the nanomolar to picomolar range. Thus far, we have selected aptamer probes for many different diseases, and used them to carry out studies at the vanguard of biomedical science, including ultrasensitive detection of tumors, molecular imaging, targeted drug delivery, and, most critically, cancer biomarker discovery. Taken together, these molecular level tools form a solid scientific platform from which to pursue advanced studies in molecular medicine. We will report our most recent progress in this exciting research area, especially the molecular elucidation of cancer biomarkers and targeted drug development.
Nucleic Acid Aptamers (NAAs) are a class of molecules with significant potential in developing molecular tools in biomedical applications. Aptamers are selected using a screening method called Systematic Evolution Ligands by EXponential enrichment (SELEX). Recently, a number of SELEX approaches utilizing whole cells to evolve aptamers against cell-surface membrane proteins were introduced. To this end, we reported on a new variant of SELEX termed Ligand-Guided-Selection (LIGS) to identify highly specific aptamers against a predetermined epitope of a cell-surface target. Hallmark of LIGS rooted in the ability of LIGS to exploit the evolutionary selection step in SELEX as a strategy to evolve highly specific aptamers. The iterative process in conventional SELEX is designed to outcompete low-affinity binders through a competitive process whereby high affinity binders move on through the selection process. By introducing a naturally occurring stronger, highly specific bivalent binder, for example, an antibody (Ab) interacting with its cognate epitope, LIGS out-competes specific aptamers from a partially enriched SELEX pool against cells expressing the same epitope. Utilizing LIGS we have selected aptamers against membrane bound IgM (mIgM) expressed on B-cells and Cluster of differentiation 3 (CD3) expressed on T-cells. Based on the detailed validation studies conducted using one of the aptamers selected using LIGS, we will show that aptamers identified using LIGS can be optimized into higher affinity variants.

**Keywords:** Biological Samples, Biomedical, Biopharmaceutical, Biotechnology

**Application Code:** Biomedical

**Methodology Code:** Chemical Methods
Development of Thioaptamers, X-Aptamers, and Associated Software

Using both sulfur substitutions for stability and protein-like or drug-like side chains, DNA aptamers with enhanced binding strengths and serum stability can be created for specific targeting. Such aptamers can be used to down-regulate a specific protein target by direct interference, or be used as a delivery agent to guide siRNA-bearing liposomes or nanoparticles to the desired cells. Some of our recent work in this area will be discussed.

Keywords: Bioinformatics, Biotechnology, Combinatorial Chemistry, Nucleic Acids
Application Code: Biomedical
Methodology Code: Chemical Methods
Advances in Nucleic Acid Aptamers as Molecular Tools in Nanotechnology and Theranostics Development

Tunable Cytotoxic Aptamer-Drug Conjugates for the Treatment of Prostate Cancer

Therapies that can eliminate both local and metastatic prostate tumor legions while sparing normal organ tissue are desperately needed. With the goal of developing an improved drug targeting strategy, we turned to a new class of targeted anticancer therapeutics: aptamers conjugated to highly toxic chemotherapeutics. Selection for aptamers with prostate cancer specificity yielded the E3 aptamer, which internalizes into prostate cancer cells without targeting normal prostate cells. Subsequent chemical conjugation of E3 to the highly toxic drugs MMAE and MMAF (at a 1:1 aptamer:drug ratio) efficiently kills prostate cancer cells in vitro but does not affect normal prostate epithelial cells. Importantly, the E3 aptamer targets tumors in vivo and treatment with the E3-MMAF conjugate significantly inhibits prostate xenograft growth in mice, demonstrating the in vivo utility of aptamer-drug conjugates. Additionally, we report the use of antidotes to reverse E3 aptamer-drug cytotoxicity, providing a safety switch in the unexpected event of normal cell killing in vivo.

Keywords: Biopharmaceutical, Fluorescence, Nucleic Acids
Application Code: Biomedical
Methodology Code: Chemical Methods
Our laboratory at MIT has been interested in exploring the relatively new interface between living plants and non-biological nanostructures to impart the former with new and enhanced functions, which we call Plant Nanobionics. We demonstrate that the introduction of particular nanoparticles such as poly (acrylic acid) nanoceria (PAA-NC) and their corresponding nanotube complexes (SWNT-NC) into isolated chloroplasts can yield chloroplasts that are more stable to reactive oxygen species ex vivo1,2, possess enhanced solar conversion efficiencies, are able to produce glucose, and allow real-time information exchange via embedded nanosensors for free radicals. Specifically, superoxide concentration can be suppressed 59% by assembling PAA-NC inside chloroplasts. SWNT-NC promote photoactivity 3.1 times above controls while PAA-NC extend glucose production from 0.5 to 88 hours2. SWNT chloroplast complexes also allow fluorescent reporting of nitric oxide generation. We further investigate the transport and localization of nanoparticles into plant cells (protoplasts) and organelles (chloroplasts) and show that strongly cationic or anionic nanoparticles (| zeta potential | > 30 mV) are able to penetrate and remain kinetically trapped within chloroplasts. A generalized Lipid Exchange Envelope Penetration (LEEP) mechanism3 for nanoparticle localization within living plants was developed with excellent predictive capabilities on what particle sizes and types will localize within plant tissue, and should be a valuable tool for plant nanobionic engineering. Lastly, progress toward photonic plants and those capable of sensing4, IR communication, and groundwater monitoring in real time will be described. Plant Nanobionics has potential to create new technology using wild-type, living plants as the starting platform.

Keywords: Nanotechnology
Application Code: Nanotechnology
Methodology Code: New Method
Citrus greening disease, also known as Huanglongbing (HLB), is posing a worldwide threat to the multi-billion dollars citrus industry. Containment of the disease is heavily dependent on early detection of infected hosts for quarantine. A major pathogen responsible is the bacteria Candidatus Liberibacter asiaticus (CLas). Current HLB detection methods are based on qualitative assessment of disease symptoms and nucleic acid assays that are susceptible to error and inaccuracies due to variable latent time and sporadic distribution of the pathogens in infected plants.

We report two nanosensors based on semiconducting single-walled carbon nanotubes (sSWNTs) chemiresistor for the rapid, facile, low cost onsite detection of HLB infection in citrus. The first sensor detects an antigen secreted by CLas in the tissue phloem by sSWNTs chemiresistor transducer functionalized with antibodies specific to the secreted antigen. Antigen-antibody binding at the surface of sSWNTs lead to changes in the local electrostatic environment and consequently to proportional modulation of electrical resistance of the sensing device. The immunosensor successfully detected HLB biomarkers at nanomolar concentration with high selectivity against other biological compounds in different citrus trees phloem extract.

The second sensor detects a signature blend of five most discriminating volatile organic carbons (VOCs) released by the infected plant associated with the asymptomatic stage of HLB. The sensor consists of an array of sSWNTs chemiresistor transducers functionalized non-covalently with different metalloporphyrins (MPs). The adsorption of the VOCs on the sensor causes change in the electrical properties of carbon nanotubes. By monitoring the change in device resistance as a function of VOC concentration, combined with enhancement of selectivity achieved by using different central metal ions in MPs, we selectively detected low concentrations of VOCs in a simple, cost-effective manner.

Keywords: Agricultural, Biosensors, Detection, Volatile Organic Compounds
Application Code: Agriculture
Methodology Code: Sensors
All living things produce metabolites that we can measure, and some of these compounds are in the gas phase as volatiles. Plant systems off gas volatile organic compounds (VOCs) that have been shown to associate with both biotic and abiotic stress. By tailoring new sensor platforms that are low size, weight and power (SWAP), we can integrate VOC measurements into both automated land and air vehicles. Coupling sensors with unmanned aerial systems (UAS) allows wide acreage coverage for potentially low cost high throughput crop health monitoring.

Keywords: Metabolomics, Metabonomics, Volatile Organic Compounds
Application Code: Agriculture
Methodology Code: Sensors
Economic losses to agriculture due to pest and pathogen infections are estimated at $40 billion annually to the agricultural industry. Early detection of pest or pathogen infection in agricultural crops through reliable detection of disease symptoms could help in improved crop management practices such as selective and timely application of fungicides thereby reducing the cost of spraying in the crop field which in turn leads to improved food quality and reduced environmental footprint of pesticides and fungicides. There is a pressing need to develop rapid, highly selective and sensitive detection technologies for early identification of plant pathogen infections. While a variety of molecular methods are currently being used for this purpose, an inexpensive, high selective, rapid method for the detection of pathogens is highly desired. Electrochemistry biosensors offer unique advantages to this application. Electrochemical sensors have been widely explored for medical and environmental sensing applications, but not as much for agricultural applications. An electrochemical biosensor uses a highly selective bio-recognition element such as enzymes, antibody, aptamer or virus and is capable of detecting binding events with ultra-low detection limits. This presentation will focus on some of the recent developments in our lab in the development of electrochemical biosensors for detection of crop diseases.

Keywords: Agricultural, Nanotechnology, Sensors, Voltammetry
Application Code: Agriculture
Methodology Code: Sensors
Over the last 15 years, our group has been engineering DNA as both genetic (bio-) materials and generic (nano-) materials. We have designed DNA as polymers in order to develop bulk-scale, DNA-based biomaterials for real-world applications including on-site diagnosis of plant pathogens. More specifically, we have created branched DNA as nanoscale barcodes for the detection of pathogen DNA. DNA signals were sequentially amplified either enzyme-free (via DNA aggregations) or by using a room-temperature polymerase with specially designed primers. For the readout, we employed microfluidics to miniaturize the detection process. Recently we have discovered a novel way to generate DNA patterns only in the presence of pathogen nucleic acids. These patterns can further be cognitively recognized with smart phones, greatly increase the signal/noise ratio of the detection. DNA have proven to be not only the molecule of life, but also the molecule for diagnosis.
Applications of Low Frequency Raman Spectroscopy

Two-Dimensional Terahertz Raman Correlation Spectroscopy Study of Melt Crystallization of PHBHx Copolymer Bioplastics

Isao Noda
University of Delaware

Co-Author(s)  Brian Sobieski, Bruce Chase, John F. Rabolt

The low-frequency Raman spectral region was used to study the evolution of crystalline state of poly[(R)-3-hydroxybutyrate-co-(R)-3-hydroxyhexanoate] or PHBHx copolymer, which is a totally biodegradable plastic produced by inexpensive microbial fermentation of vegetable oils. Raman bands associated with the intermolecular lattice vibrations of crystalline lamellae were detected in the very low frequency/THz region. Their systematic intensity variations during the melt crystallization of PHBHx with decreasing temperature were contrasted to those of carbonyl vibrations using two-dimensional (2D) Raman correlation spectroscopy. Coordinated dynamic variations in the spectral features were observed with the crystallization process, and surprisingly detailed mechanisms for the development of crystalline structures were revealed.

Keywords: Material Science, Polymers & Plastics, Raman Spectroscopy, Spectroscopy

Application Code: Polymers and Plastics
Methodology Code: Vibrational Spectroscopy
Until recently very low frequency Raman measurements (5-150 cm⁻¹) required large, triple monochromator systems. However, since the introduction of volume Bragg gratings (VBG’s) measurements can be made down to 5 cm⁻¹ from the Rayleigh line on single monochromator systems. Such very low frequency Raman measurements can be performed on a variety of Raman systems including low cost macro systems, process Raman systems using optical probes, and Raman microscopes for both single point and Raman imaging applications. Important applications include layered semiconductors, active pharmaceutical ingredient (API) crystal phase, and longitudinal acoustic modes (LAM’s) in polymers. All of these materials are ordered phases where orientation can play a role, and can be explored in the polarization-dependent behavior of the Raman spectra which will be shown.

Keywords: Material Science, Pharmaceutical, Raman Spectroscopy

Application Code: Material Science

Methodology Code: Vibrational Spectroscopy
Applications of Low Frequency Raman Spectroscopy

[i]In Situ[/i] Monitoring of Solid Form Transformations

Many pharmaceutical solids can exist in different forms, e.g. crystalline and amorphous. Hence, there is a need to be able to monitor physical form during the development of drug products. There are a number of techniques that are commonly used for solid-state analysis including x-ray powder diffraction (XRPD), solid-state NMR spectrometry and infrared (IR) spectrometry. However, some of these techniques are expensive (e.g. XRPD and NMR) and most cannot be used [i]in situ[/i]. While Raman spectrometry can be used for [i]in situ[/i] structural and chemical analysis, it is the low frequency (<200 cm$^{-1}$) region in the Raman spectrum that is most sensitive to solid form. This region is generally not observed in traditional Raman measurements owing to limitations with the notch filters used to block the Rayleigh scattered light. However, major advances in ultra narrowband filters and wavelength stabilised lasers have now made the so-called THz Raman region accessible. Therefore, in this work, a novel portable THz Raman instrument has been used for [i]in situ[/i] analysis of solid form transformations in sulfamerazine.

Sulfamerazine is a widely used sulfonamide antibacterial drug and has three known polymorphic forms. Form I can be transformed to form II by milling at room temperature and to an amorphous form by cryo-milling. THz Raman spectrometry was used to determine the optimum cryo-milling time for production of the amorphous form and to study its subsequent transformation to forms I and II over a period of approximately 100 minutes. In addition, it was shown that form I transforms to form II via an amorphous form when milled at room temperature. The concentrations of the different solid forms was determined from the THz Raman spectra using a classical least squares model. The advantages of using THz Raman spectrometry will be discussed.

Keywords: Monitoring, Pharmaceutical, Process Analytical Chemistry, Raman Spectroscopy

Application Code: Pharmaceutical

Methodology Code: Vibrational Spectroscopy
Low-frequency Raman spectroscopy is a technique that has recently been applied for solid state studies.[1] For pharmaceuticals, it is very important to be able to control and monitor crystallinity, because the solid state structure can determine the bioavailability, stability, and manufacturing aspects of a drug. The advantage of low-frequency Raman spectroscopy is that it gives additional information on the lattice vibrations of a crystal which cannot be collected by the conventional, mid-frequency Raman spectroscopy. The resulting distinct spectral features between different solid state forms have been used for characterizing polymorphs of several pharmaceuticals.[2]

As an example, Low-frequency Raman spectroscopy was able to differentiate the three piroxicam solid state forms. When combined with multivariate analysis, both low-frequency and mid-frequency Raman spectra could separate all mixtures from each other. The amount of each solid state form could be predicted with all models based on the different Raman techniques and spectral regions. When comparing the low and mid-frequency spectral data collected using the same Raman instrument (Figure 1), models built with the low-frequency data performed slightly better. This implies that there is an advantage in using low-frequency over mid-frequency Raman data. The signal strengths were higher at the low-frequency range, which offers potential advantage of better signal-to-noise ratio.

Figure 1. Left panel: Solid—state low frequency Raman spectra for differing forms of piroxicam. Right panel: Superposition of the tertiary mixtures of the three forms of piroxicam (orange dots) and the principal components analysis from the low frequency data.


Keywords: Pharmaceutical, Polymers & Plastics, Raman Spectroscopy, Vibrational Spectroscopy

Application Code: Material Science

Methodology Code: Molecular Spectroscopy
Mass spectrometry based proteomics is the de-facto tool for exploratory analysis of proteins and data from these large-scale studies can be used to create hypothesis about biochemical mechanisms related to diseases and other biological states. To further explore these hypothesis, however, detailed information about these proteins must be acquired, including interaction network and structure of proteins and their respective complexes. Here, the structural biology plays a vital role by solving the structure of proteins and complexes, creating the possibility of engineering molecules and designing proteins to modulate specific responses. Besides the large number of structures solved by structural biology using X-Ray diffraction (along with nuclear magnetic resonance and most recently cryo-microscopy), most proteins are not amenable to these techniques and new methods to gather structural information of proteins and complexes are needed. In this talk, we will show the advances in mass spectrometry based structural biology (structural proteomics) in revealing the structure and dynamics of proteins and how this information can be used to devise mechanisms of biological processes.

Keywords: Bioanalytical, Mass Spectrometry, Protein, Proteomics
Application Code: Genomics, Proteomics and Other Omics
Methodology Code: Mass Spectrometry
Metabolomics has become a dominant approach within the systems biology paradigm, that imposes a holistic view of living systems via mathematical models, data integration, and physiological measurements, relying on information that flows through an omics cascade, from genes to transcripts, proteins and finally their expression, the metabolites, and the intricate relationships among them. The collective set of metabolites (metabolome) can be interpreted as the downstream result of implementation of genomic information, although environmental modulation plays quite a vital role. Therefore, metabolomics is thought to be a promising generator source of large-scale phenotyping data, that will ultimately help bridging the gap between genotype and the acquired phenotype, and improving the knowledge of their complex dynamics.

From a practical perspective, metabolomics can be defined as the quali- or quantitative analysis of the entire set of metabolites expressed by a living system, in pre-established conditions, via comparative experiments, which can be fairly easily implemented nowadays due to the remarkable advances of NMR- and MS-based analytical platforms and elegant bioinformatics tools. Both formats are possible, untargeted (hypothesis generating) and targeted (hypothesis driven) metabolomics, and many relevant applications can be envisioned.

This work will describe our efforts towards implementation of a metabolomics facility in our institution. Early findings using targeted approaches (THEN) in clinical (diabetes related metabolites) and forensic (cocaine abuse) applications, and more recent untargeted clinical metabolomics studies (NOW), describing the efficacy testing of novel natural-product derived drugs for the treatment of leishmaniasis, effects of air pollution and gestational use of marijuana on fetal outcomes, and the contrasting of immunosuppressive therapeutical regimens for kidney transplanted patients, will be discussed.

Acknowledgements: FAPESP, CNPq

Keywords: Clinical/Toxicology, Metabolomics, Metabonomics
Application Code: Clinical/Toxicology
Methodology Code: Gas Chromatography/Mass Spectrometry
Proteomics has been performed in Brazil long before the term "proteomics" was coined in 1994. It was a result from efforts undertaken by some protein chemistry groups since the 1960’s, leading to the full determination of a protein sequence by Prof. Lauro Morhy at the University of Brasilia (UnB) in the 1980’s. By that time, 2D electrophoresis was already done routinely there, but protein identification used to rely on automated chemical sequencing. In early 1990’s, I and colleagues at UnB pioneered protein mass spectrometry in Brazil. Currently, proteomics is a widespread discipline in Brazil. More than forty groups are established in the country states. The most traditional groups are in the states of São Paulo and Rio de Janeiro as well as in the capital Brasilia. Essentially all the fashions of proteomic techniques are now performed in Brazil, from conventional 2DE up to modern top down proteomics. Most of the current works are directed to quantitative proteomics. In addition, there are also researchers succeeding with SRM-MS, HDX-MS and other applications. Concerning mass spectrometers dedicated to proteomics, Brazil has over one hundred of them, ranging from old time-of-flight up to new, high end Orbitraps. Again, most of this equipment arsenal is located in Brasilia, São Paulo and Rio de Janeiro, but most of the other states also have theirs. Proteomics is used to solve problems in a plethora of areas in biology, biotechnology, pharmaceutics, medicine and agriculture in Brazil. Regarding formal institutional organization, in 2012, we set up the Brazilian Proteomics Society (BrProt) under the leadership of Prof. Gilberto Domont. There is a movement within BrProt to host a HUPO annual meeting in Brazil in the near future. We envisage that proteomics in Brazil will keep growing both quantitatively and qualitatively. Surely, new equipment, software, approaches, scientific questions and people will be incorporated to the Brazilian proteomics in the years to come.

Keywords: Bioinformatics, Mass Spectrometry, Protein, Proteomics
Application Code: Genomics, Proteomics and Other 'Omics
Methodology Code: Mass Spectrometry
Development of Omics Sciences in Brazil

Metallomics-Based Systems Applied to Biological Systems

Metallomics was recently coined to describe integrated biometal science, in symbiosis with genomics and proteomics, because syntheses and metabolic functions of genes (DNA and RNA) and proteins cannot be performed without the aid of various metal ions and metalloenzymes [1]. In this sense, the metallome is defined as metalloproteins, metalloenzymes and other metal-containing biomolecules, in a similar way to genomes in genomics as well as proteomes in proteomics [2-4]. The metallomic information comprises the identities of the individual metal species (qualitative metallomics) and their concentrations (quantitative metallomics). Then, and inside this context, our contribution to metallomics is pointed out through some examples involving forensic, plants/seeds and diseases, characterizing the integration of this science with others.


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Keywords: Bioanalytical, Food Science, Forensics, Mass Spectrometry
Application Code: Bioanalytical
Methodology Code: Mass Spectrometry
Next Generation Separations Technology and Chemistry

Enhancing the Effective Peak Capacity for Multidimensional Gas Chromatography through Advances in Instrumentation and Chemometrics

Robert E. Synovec
University of Washington

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Abstract Title: Enhancing the Effective Peak Capacity for Multidimensional Gas Chromatography through Advances in Instrumentation and Chemometrics
Primary Author: Robert E. Synovec
University of Washington

Abstract Text

GC – TOFMS and GC x GC – TOFMS have emerged as powerful chemical separation platforms for the analysis of complex samples. Advances in the technology have come in many arenas: implementing chemical separations theory to provide insight into producing higher peak capacity separations, new and improved instrumentation designs, and chemometric software developments for peak deconvolution to effectively increase the peak capacity. Each of these arenas couple with, and contribute to, advances in the other arenas. Recent instrumentation developments for high efficiency GC and GC x GC are based upon applying sound theoretical understanding to optimize peak capacity production, and then experimentally producing data that exhibits the optimized performance. We are developing new ways to analyze GC – TOFMS data which radically improve the “chemometric-enhanced” peak capacity, resulting in improved analyte identification with superior quantitative accuracy and precision. With GC x GC – TOFMS, we are developing new instrumental designs that simultaneously increase the chemical selectivity provided by the separation while increasing peak capacity production. Furthermore, these advances in GC x GC – TOFMS instrumentation design are integrated with chemometric deconvolution strategies to optimize the generation of useful chemical information.

Keywords: Chemometrics, Data Analysis, Gas Chromatography/Mass Spectrometry, Time of Flight MS
Application Code: General Interest
Methodology Code: Gas Chromatography/Mass Spectrometry
Dielectrophoresis (DEP) allows to exploit the polarization properties of bioparticles and even biomolecules for migration and trapping applications. The methods thus adds an additional parameter space to separation and fractionation applications. Among the various realizations of dielectrophoretic devices, insulator-based DEP (iDEP) can be realized with simple microfabrication tools using elastomers, does not require micropatterning of electrodes and allows applications in the low frequency range. Electric fields are generated in iDEP devices by applying potential to the extremities of a microfluidic device while insulating structures are embedded to create inhomogeneous electric fields necessary to generate dielectrophoretic response of analytes. Utilizing this approach, we have designed a constriction sorter for fractionating protein crystals by size. Novel serial crystallography applications with free electron lasers require defined small and size homogeneous crystals for improved protein structure determination. We have successfully developed microfluidic constriction sorters, capable of fractionating protein crystals by size for crystallography applications. Further, we have employed constriction and array-based sorters for fractionation of kbp DNA by size. The latter is important for novel next generation sequencing applications requiring tens of kbp DNA for optimum sequencing readout. In addition, the development of a tunable iDEP device will be presented, realized through deflecting membranes in an elastomer device.

Keywords: Bioanalytical, Lab-on-a-Chip/Microfluidics, Separation Sciences
Application Code: Bioanalytical
Methodology Code: Separation Sciences
Many proteins are attractive therapeutic targets while small molecules that can selectively and strongly bind them are potential drug leads. Label- and immobilization-free screening of strong protein-small molecule binding is pivotal for high-throughput drug development. Here, we introduce longitudinal separation by transverse diffusion in laminar pipe flow (LSTDLPF): an immobilization-free approach for finding the equilibrium dissociation constant (Kd) of strong reversible binding of protein and small molecule. When combined with mass-spectrometric (MS) detection, the approach is also label-free. Conceptually, a short plug of the equilibrium mixture comprising a protein, a small molecule, and small molecule-protein complex is injected into a capillary tube and propagated by pressure-driven buffer solution under laminar flow conditions. The unbound small molecule is separated from the protein-bound one by transverse diffusion based on differences in diffusion coefficients of the small molecule and the protein-small molecule complex. A signal proportional to the concentration of the small molecule, both unbound and bound to the protein, is measured and Kd is calculated based on the measured signal-versus-time dependence. We developed instrumentation for LSTDLPF and proved LSTDLPF in principle by measuring Kd for 2 protein-small complexes with both fluorescence and MS detection. We foresee that LSTDLPF has a potential to become a practical tool for studying protein-small molecule binding for binding pairs which are difficult to study with classical biosensoric or calorimetric approaches.

Keywords: Bioanalytical, Drug Discovery, Liquid Chromatography
Application Code: Drug Discovery
Methodology Code: Liquid Chromatography
Polyethylene terephthalate (Pe; overhead transparencies) can be exploited as the substrate for microfluidic devices with printer toner (T) used as adhesive to generate rotation-driven microdevices (RDMs) for a variety of analytical processes. We demonstrate that CD-like PeT microchips can be fabricated for fluidic transport controlled by centrifugal forces and valving by laser-printed hydrophobic valves or laser actuated physical valves. By simply using rotation speeds that generate enough centrifugal force, fluids could be mobilized through open channels and, at higher speeds, through hydrophobic valves. In a PeT RDM with two fluidic layers fabricated by laser ablating the microfeatures in the Pe sheet and then laminating using the toner for bonding, we show that a simple colorimetric protein assay can be carried out in an automated manner. In doing so, we highlight the ability to integrate a substantial number of fluidic control elements to achieve a basic diagnostic functionality (i.e., protein quantification). The functionality requires fluid metering, mixing, and aliquoting, and thus, represents an effective demonstration of fundamental operations required for a broad range of other applications. Exploiting the ability for smartphone colorimetric analysis, we will discuss the ability to detect explosives and illicit drugs on a simple spin-driven device. In addition, we will demonstrate the integration ability of these systems by discussing a genetic analysis system that generates DNA profiles from buccal swabs. Fusing the cost-effective and versatile PeT RDM with a simple detection modalities presents a promising strategy for automation and multiplexing of chemical and biochemical assays for a variety of applications.

Keywords: Bioanalytical, Biological Samples, Biosensors, Genomics
Application Code: Bioanalytical
Methodology Code: Microfluidics/Lab-on-a-Chip
Liquid chromatography (LC) is the method of choice for separating a great variety of mixtures that contain non-volatile analytes or matrix components. New instrumentation has heralded the progress from high-performance LC to “ultra-high-performance” LC. However, UHPLC does not suffice to separate complex samples. For samples containing 100 or more components long columns, slow gradients and relatively large packing particles or monolithic columns are often used and analysis times are often long.

For such complex samples comprehensive two-dimensional LC (LC×LC) presents an attractive alternative. It offers much greater peak capacities in a much shorter time. In addition, LC×LC provides additional selectivity and possibly structured, readily interpretable chromatograms. LC×LC is relatively easy to perform, but some important issues must be addressed. In principle, the effluent of the first-dimension column is the injection solvent for the second-dimension separation. This may give rise to (in-) compatibility issues. These may be addressed by “active modulation”, for example by inserting traps in the modulation value.

Method-development is another obstacle to the proliferation of LC×LC. Because the process is considerably more difficult than for 1D-LC and because the benefits of successful method development and optimization (in terms of analysis time and separation quality) are much greater, the development of smart software programs, such as PIOTR, is of great practical importance.

Finally, the promise of spatial LC for separating extremely complex samples. Ultimately, spatial three-dimensional LC promises peak capacities of the order of one million. To achieve this, analytes will be separated “in space” (i.e. based on their x-y coordinates after the first two separations), followed by elution in the z-direction.
Highly sensitive, robust, and ultimately simple technologies for converting volatile and nonvolatile compounds to gas-phase ions at room temperature and even under freezing conditions without the need of a laser, high voltage, or particle bombardment have been reported. Ionization spontaneously occurs upon exposure of the matrix:analyte sample to conditions that evoke sublimation/evaporation of the matrix which is a solid in matrix-assisted ionization (MAI) or a liquid in solvent-assisted ionization (SAI). Simply mixing the analyte with solid matrix as in MALDI or a solvent as in ESI and exposure to the vacuum of the mass spectrometer produces ions of the analyte. The matrices are as volatile as those used in ESI and much more so than MALDI all but eliminating matrix related source contamination. The limits of quantitation with these methods are low attomoles of analyte and clean full-acquisition mass spectra have been obtained with only a few femtomoles. While MAI, like MALDI, is used for direct analysis of samples, but on common atmospheric pressure mass spectrometers without a laser, SAI can be coupled with LC/MS for separation prior to analysis. These new ionization processes can be coupled with ion mobility for nearly instantaneous additional gas-phase separations. Applying a voltage in SAI provides similar mass spectra and selectivity as ESI, but at 3 to 10X higher ion abundance. Fundamentals of these novel ionization processes will be discussed, but primarily the presentation will focus on analytical applications and both manual and automated analyses of real samples. These include characterization, without any sample clean-up, of drugs in urine, proteins in cells and blood, metabolites from tissue and urine, and peptides from protein digests.

Keywords: Biological Samples, Liquid Chromatography, Mass Spectrometry
Application Code: Bioanalytical
Methodology Code: Mass Spectrometry
Molecular composition analysis is essential to our understanding of aerosol evolution and growth in the atmosphere, but typically can only be done offline, sacrificing time resolution. This creates a need for an analysis method capable of performing molecular analysis online. Droplet Assisted Inlet Ionization (DAII) has been developed for this purpose. In DAII, submicron particles are first grown into aqueous microdroplets. The droplets subsequently pass through a heated capillary tube, where rapid vaporization results in the formation of molecular ions. Initial development of DAII has been done primarily with poly(propylene)-glycol (PPG) oligomers. DAII can detect PPG particles as small as 13nm in diameter, with sampled masses on the order of picograms. Additionally, particle and mass concentrations as low 1000 particles/cm³ and 100 ng/m³ respectively can be measured, surpassing the necessary detection limits required to make ambient measurements. Further studies have been done with bovine serum albumin and angiotensin to demonstrate the capabilities of DAII to induce multiple charging on a single analyte, making DAII sensitive to both large and small molecules. Oligomers in secondary aerosol derived from cyclic siloxane oxidation have also been studied, with high resolution and good signal-to-noise in both positive and negative ion modes. These plus future studies of DAII will be presented.

Keywords: Aerosols/Particulates, Environmental Analysis, Mass Spectrometry, Nanotechnology

Application Code: Environmental

Methodology Code: Mass Spectrometry
Matrix-assisted ionization (MAI) is a novel method for ion formation that is simple and robust and is able to create multiply-charged ions from large biomolecules by the application of a mechanical shock. The application of MAI to mass spectrometry imaging poses the technological challenge of spatially and temporally localizing the formation of ions. We are developing methods for pulsed MAI that use a focused shockwave to produce ions under ambient conditions. The application of a focused local shockwave force has the potential to add to the imaging capabilities of MAI and significantly increase its utility in this regard. In our initial implementation of a local shockwave, a high speed pulsed valve was used to direct a burst of gas at a thin foil upon which an inlet ionization sample had been deposited. With the electrically activated pulsed valve, samples were ionized with high temporal and spatial precision before introduction into an ion trap mass spectrometer. Here, a pulsed valve was mounted 1 mm behind a 25-mm diameter suspended metal foil. The valve has a 500 µm orifice and was used with a backing pressure of 600 kPa. It was located 1 cm away from the mass spectrometer inlet. For dried droplet-prepared protein standards, the shockwave-generated ions were observed 1s after the pulse and the signal decayed within a few seconds. Piezoelectric and electrohydraulic shockwave sources are also being developed to achieve precise spatial as well as temporal focusing. A piezoelectric element with a narrow striker is placed behind the foil and actuated with a voltage controller. Alternatively, a spark in liquid is focused by an elliptical reflector directed at the sample target. The goal is to submillimeter imaging resolution on thin tissue sections mounted on metal targets.

Keywords: Bioanalytical, Biological Samples, Biomedical, Mass Spectrometry
Application Code: Bioanalytical
Methodology Code: Mass Spectrometry
Because of its selectivity and high sensitivity, mass spectrometry has become an important technique in the determination of proteins in biological matrices. Most commonly the bottom-up approach is used in which the proteins are digested to peptides prior to separation and detection by liquid-chromatography mass spectrometry (LC-MS) using electrospray ionization (ESI).

Recently matrix assisted ionization (MAI) and solvent assisted ionization (SAI) were introduced as new ionization techniques for mass spectrometry, both able to produce ESI-like ions from either a solid or solution matrix without voltage. It has previously been demonstrated that MAI and SAI can be applied for ionization of intact and digested proteins using various formats.

In the presented work, the use of MAI and SAI in targeted protein determination using a triple quadrupole MS will be discussed. Proteins are important analytes to monitor in a clinical setting due to their use as disease markers, drugs and doping agents. Both established model proteins and relevant biomarkers are used as model compounds. Analysis are performed from both standard solutions and biological matrices. The advantages and disadvantages of these new ionization methods will be discussed relative to conventional ionization methods.

Keywords: Bioanalytical, Monitoring, Sample Introduction, Tandem Mass Spec
Application Code: Clinical/Toxicology
Methodology Code: Mass Spectrometry
Powerful New Ionization Processes in Mass Spectrometry

Spontaneous Charge Separation Processes in the Inlet Tube of a Mass Spectrometer

Charge separation is a necessary route in the ionization process of a mass spectrometer. Most of the ionization technique apply additional energy as photons, heat, particle bombardment, or electrical potential to create the gas-phase ions necessary for analysis using mass spectrometry (MS). Unlike the conventional methods, some matrices used with matrix assisted ionization (MAI) and solvent assisted ionization (SAI) provide both simplicity and high sensitivity without the necessity of applying energy. The solid or liquid matrix:analyte samples are typically introduced into and pass through an inlet tube from a higher to a lower pressure region to generate the analyte ions. Previous studies have demonstrated that these novel ionization techniques show impressive analytical capabilities in various fields from forensics to pharmaceuticals. However, the detailed ionization mechanism still remains a mystery. Here we present systematic studies to monitor the charge formation processes in order to determine possible driving forces of these novel ionization processes. Combining a set of capillary and charge detectors, a home-built inlet ionization source simulates the conventional inlet conditions of a mass spectrometer. The charge generated by positive and negative particles can then be observed at various pressure differences and gas flow across and through the inlet tube. The experimental results depict the formation of charge particles and provide clues to the driving force and ionization mechanism as well. A detailed understanding of this spontaneous charge separation process will shine a light on the development of next-generation ionization techniques in mass spectrometry.

Keywords: Mass Spectrometry, Materials Characterization, Temperature

Application Code: High-Throughput Chemical Analysis

Methodology Code: Mass Spectrometry
Precompetitive Collaborations on Enabling Technologies for Pharmaceutical Research and Development

Enabling Technologies Consortium (ETC): Leveraging the Power of Numbers in the Development of “Next Generation Supercritical Fluid Chromatography (SFC) Instrumentation”

Preparative SFC has been a preferred method for small molecule purification in the pharmaceutical industry for about 15 years, since the prep SFC instrument was introduced by the now defunct Berger Instruments. Since then several vendors have entered and exited the prep SFC marketplace and a number of commercial instruments are currently available. However, these prep SFC instruments have some performance and dependability limitations, and are generally considered to be inferior to the Berger Multigram II, a ‘gold standard’ for semi-preparative SFC purification that is no longer manufactured. Consequently, pharma companies interested in the planned replacement of aging instrumentation are uncertain how to proceed. A cross-pharma collaboration of SFC users has been formed under the auspices of the Enabling Technologies Consortium (ETC) to leverage the power of a common voice to develop the next generation of SFC instrumentation that satisfies the hardware, software and safety requirements of the user group. This presentation will highlight our experiences in collaborating through the ETC and provide details regarding the progress of the project.

Keywords: Chromatography, Prep Chromatography, Supercritical Fluid Chromatography
Application Code: Pharmaceutical
Methodology Code: Supercritical Fluid Chromatography
### Session Title
Precompetitive Collaborations on Enabling Technologies for Pharmaceutical Research and Development

### Abstract Title
A Collaborative Study on High Throughput Solid Dispensing Platforms

### Primary Author
Matthew N. Bahr
GlaxoSmithKline

### Co-Author(s)

### Abstract Text
The task of dispensing defined amounts of solid powders is an increasingly important task in Pharmaceutical R&D, especially in the area of High Throughput Experimentation (HTE). The HTE working group is a member of the Enabling Technologies Consortium (ETC), and is focused on experimenting with several solid dispensing robotic platforms in order to assess their ability to accurately weigh powder into vials, using a wide range of powders and under a variety of lab environments. In this presentation, we will discuss the statistical outcome of more than 18,000 data points that were collected at five pharmaceutical companies on these instruments over an eight month period of time. The results of this analysis have led the group to conclude that there are several crucial factors that affect the performance of powder dispensing.

### Keywords
Laboratory Automation, Pharmaceutical, Sample Handling/Automation, Statistical Data Analysis

### Application Code
Pharmaceutical

### Methodology Code
Sampling and Sample Preparation
Shedding a Light on Solubility Measurement and Prediction – The ETC Solubility Sub Team’s Story to Date

Understanding the solubility of organic compounds in organic solvents is a fundamental task in drug development. Over the last decade many in the industry have begun to seek out others to help understand fundamental issues that are common throughout the industry. The Enabling Technologies Consortium (ETC) solubility team is testament to this collaborative spirit and this presentation will detail the results of our collaborations to date. This talk will highlight how representatives from the ETC member companies have worked together to: 1) better the measurement and prediction of solubility data through sharing of current practice from both the predictive and experimental sides; 2) defined a common testing program; and 3) analysed these data to define the best practice.

A common objective between the members of this collaboration is that we can chose the best solvent for our reactions, workups, isolation and even plant cleaning. The compounds may differ but the technological desire does not. From these experimental data we hope to be able to accurately predict the solubility of hundreds of single solvents and innumerable solvent combinations. Ultimately we all desire the same thing. An easy to run, reliable, accurate and precise method for measuring the solubility of numerous APIs and drug intermediates.

The next steps for this team include publication of these finding as a prelude to the ultimate goal to partner with vendors to help define the next generation of equipment and software tools.

Keywords: Chemometrics, Laboratory Automation, Quantitative, Robotics
Application Code: Pharmaceutical
Methodology Code: Physical Measurements
Precompetitive Collaborations on Enabling Technologies for Pharmaceutical Research and Development

Addressing Drying Challenges in the Pharmaceutical Industry

Within the pharmaceutical industry the drying unit operation is ubiquitous in the isolation of solid intermediates and products, and is often the final unit operation in the manufacturing of drug substance. Due to the complexity of the drying process and range of equipment types and scales, the impact of process parameters on critical drug substance quality attributes is not well understood. To advance the industrial understanding of this process a Drying Working Group (WG) was formed within the Enabling Technologies Consortium (ETC), which facilitates collaborations among multiple companies to deliver innovative solutions on pre-competitive challenges. This presentation outlines how the Drying WG identified common challenges experienced during drying development, including limited material quantities for scale-up studies and transferring processes to different equipment types, and devised a strategy to address those challenges. The team is working to close three key gaps to address these challenges: process analytical technology (PAT), modeling and scale-down equipment. The current focus of the Drying WG is to explore the application of novel PAT in the drying process to gain real-time data. The eventual aim is to utilize these data to design more robust models and improved scale-down laboratory equipment that will improve the efficiency and understanding of drying process development and resulting control strategies.

Keywords: Drugs, On-line, Pharmaceutical, Process Monitoring
Application Code: Pharmaceutical
Methodology Code: Process Analytical Techniques
The Crystallization Working Group within the Enabling Technologies Consortium™ (ETC) has defined a project to develop a multi-dimensional particle size and shape measurement tool. The working group will engage with academics and vendors to develop technology that will improve our ability to model particle size and shape and predict downstream performance of powders by measuring and providing meaningful descriptors of multidimensional particles as they form and grow.

The current state of the art commercial equipment provides either a one-dimensional trending statistic (e.g. chord length) that is loosely correlated to particle size, an image analysis routine with limited multidimensional information and poor resolution, and/or off-line image analysis with slurry dilution due to inability to collect quality images in a concentrated suspension.

The primary focus of this talk will be the evaluation of existing particle imaging technologies, identification of gaps in the current commercial offerings with respect to size and shape measurement and analysis, and discussion of how the ETC members are proposing to evaluate novel technology to close these gaps. Technologies that will be discussed include FBRM, PVM, Perdix, Canty, and a proprietary imaging technique using a flow through cell.

The talk will also highlight efforts the Crystallization Working Group has made toward improving the user-friendliness of available population balance modeling tools and developing, namely improving the path from raw analytical data to fully developed models as well as our groups desire to develop improved tools for impurity rejection and morphology screening.

Keywords: Imaging, Particle Size and Distribution, Process Monitoring
Application Code: Pharmaceutical
Methodology Code: Process Analytical Techniques
Chemical forensics has been recently recognized as a new discipline that aims to obtain information from chemical remnants that is relevant to investigative, legal and intelligence questions. In our research work, the application of heated headspace solid phase micro extraction (HHS-SPME) in chemical forensics of marijuana was investigated. Marijuana is currently a Schedule I controlled substance under the federal perspective. Recent changes in some state legislation of marijuana is shifting the forensic task from chemical analysis of marijuana into the determination of marijuana varieties. Legal marijuana may be diverted from its intended use. Regardless the legal status of marijuana in the United States, there is a need to develop an effective and reliable analytical platform and database to determine the variety or source of marijuana evidence for the purpose of law enforcement and forensic intelligence. In this work, headspace chemical signature of standard marijuana samples was extracted by automated HHS-SPME. The extract was injected to a gas chromatography-mass spectrometry (GC-MS) for separation and analysis. The obtained chemical data from different varieties of marijuana were digitized and processed with ensemble learning algorithm in order to build a learning model for the intelligent classification of marijuana varieties. The successful classification of marijuana varieties using headspace chemical analysis combined with machine learning technique will be demonstrated in this presentation. The overall goal in our research group is to develop a forensic analytical system with advanced machine learning technology that is more efficient, robust which will benefit and strengthen the practice of chemical forensics in controlled substance and trace evidence analysis. Future application of HHS-SPME-GC/MS analytical platform in chemical forensics will also be discussed.

This research was funded by the National Institute of Justice (Award #2014-R2-CX-K005).

**Keywords:** Forensics, GC-MS, Headspace, SPME

**Application Code:** Homeland Security/Forensics

**Methodology Code:** Gas Chromatography/Mass Spectrometry
Innovations and Trends in Forensic Examination of Seized Drugs and Forensic Toxicology

Forensic Identification of Plant-based “Legal Highs” by Chemometric Processing of Direct Analysis in Real Time Mass Spectrometry (DART-MS)-derived Chemical Fingerprints

The past five years have witnessed a dramatic increase in the abuse of currently unscheduled plant-based psychotropics (PBPs). It continues to be impractical for crime labs to develop standard operating protocols for the myriad of new products that continually appear. We demonstrate here how DART-TOF-MS permits rapid identification of PBP evidence in its native form. Roots, seeds, and aerial plant parts can all be tested directly without the need for the customary sample extraction and other time consuming preparation steps. A single analysis enabled detection of a broad range of both polar and non-polar molecules. In addition, readily identifiable diagnostic chemotaxonomic markers were observed in each case. These included mitragynine and 7-hydroxymitragynine in Kratom, kavain, yangonin, methysticin and dehydrokavain in kava powder, and salvinorins A and B and divinatorin A in Diviner’s sage, among several others. The appearance of these compounds in supplements and other products was also useful in identifying the plants from which supplement forms of plant-based legal highs were derived.

Multivariate statistical analysis processing of the DART-TOF-MS data by various approaches including principle component analysis (PCA), linear discriminant analysis, and partial least squares discriminant analysis (PLSDA) showed that the various species were readily distinguishable based on their mass spectral fingerprints, and well-defined species-specific clustering was observed. Furthermore, testing of the classification systems with plant material unknowns resulted in correct identifications in all cases.

The speed of analysis using this ambient ionization technique dramatically reduces overall analysis time, which makes it practical to quickly generate the large data sets required for the successful application of multivariate statistical analysis to the development of databases of abused substances that can be used for forensic identification and characterization.

**Keywords:** Chemometrics, Drugs, Forensic Chemistry, Identification

**Application Code:** Homeland Security/Forensics

**Methodology Code:** Mass Spectrometry
Identification of synthetic designer drugs continues to pose challenges in forensic laboratories due to the high structural similarity among compounds in a given class and the rapid emergence of new analogs. Controlled substance identification is based on comparison of the gas chromatographic retention time and mass spectrum to that of a reference material. However, this visual comparison provides no statistical assessment of the veracity of the identification. Further, identification of new analogs is especially problematic as no reference material is available for comparison.

This presentation will describe statistical and mass spectral tools developed to aid in the identification and characterization of designer drugs. The work focused on three structural subclasses of the synthetic phenethylamines (APB-, 2C-, and NBOMe-phenethylamines), a set of which was analyzed by GC-MS. A statistical method based on the unequal variance t-test was developed for the comparison of two mass spectra. Discrimination between compounds within each phenethylamine subclass was achieved at the 99.9% confidence level, except for three NBOMe-phenethylamines which were discriminated at the 99% confidence level.

Characterization schemes were also developed to characterize compounds according to phenethylamine subclass based on mass spectral characteristics. The first scheme, based on low-resolution mass spectral data, used characteristic neutral losses to indicate the structural subclass and isotope ratios to determine the substituent. The second scheme, based on high-resolution time-of-flight mass spectral data, additionally incorporated mass defect filters to increase specificity in subclass characterization. The application of these tools for identification and characterization of synthetic phenethylamines will be demonstrated throughout this presentation.
Emerging drugs are synthetically produced alterations of the controlled drugs in order to circumvent laws banning their use as recreational drugs. The identification of existing and new emerging drugs is complicated by the similarity in structure, lack of molecular ions for certain solutes and/or similarity in mass spectral (MS) spectra for diastereomers and positional isomers and incomplete chromatographic separations. In order to increase the specificity of analysis emerging technologies such as ultra-high performance supercritical fluid chromatography (UHPSFC) with MS and ultraviolet (UV) detection, gas chromatography (GC) with vacuum ultra violet (VUV) detection and multi-dimensional ultra-high performance liquid chromatography will be discussed.

UHPSFC which provides complimentary separations to both GC and UHPLC is particularly advantageous for the separation of positional isomers and diastereomers. Peak assignments for the former technique are aided by the following: MS detection, which generates molecular ions, and UV detection, which can distinguish between classes and subclasses of emerging drugs and differentiate between many positional isomers. For UPSFC versus UHPLC there was at least a 10 nm blue shift in UV maximum. GC with VUV detection (125-240 nm wavelength interval) can distinguish most positional isomers. The use of multi-dimensional UHPLC, which provides for significantly increased peak capacity over a one dimensional separation, substantially increases the likelihood of the correct identification of an emerging drug by decreasing the uncertainty of peak assignments using retention time.

This project was supported by Awards numbers 2014-R2-CX-K009 and 2016-DN-BX-0169 awarded by the National Institute of Justice, Office of Justice Programs, and the United States Department of Justice. In addition the Perkin Elmer Corporation provided financial support for our laboratory through GWU proposal no. 13-04142.
**Session Title**
Innovations and Trends in Forensic Examination of Seized Drugs and Forensic Toxicology

**Abstract Title**
Evaluation of Drugs Other Than Nicotine (DOTNs) in an Aerosol Formed by an Electronic Cigarette

**Primary Author**
Michelle R. Peace  
Virginia Commonwealth University

**Co-Author(s)**
Alphonse Poklis, Haley A. Mulder, Joseph Turner, Justin L. Poklis, Matthew S. Halquist

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**Abstract Text**
E-cigarettes are an alternate nicotine delivery system. An aerosol is formed when the e-liquid passes over a heated coil, is vaporized, and is then condensed with water in the atmosphere. The size of the droplets formed in the aerosol can vary and the size of the particles contributes to the location where the particles will deposit in the lung. The growing popularity of these products has caused an increase in internet sources promoting the use of drugs other than nicotine (DOTNs) in e-cigarettes. DOTNs include natural and plant-based products, designer drugs, traditional and non-traditional pharmaceuticals. The purpose of this study was to compare the aerosol formed by methamphetamine and methadone to the aerosol formed by nicotine in an e-cigarette.

Methadone and methamphetamine were prepared at 60 mg/mL in 50:50 PG:VG e-liquid formulations and were aerosolized for 10 seconds into a 10-stage Micro-Orifice Uniform Deposit Impactor™ (MOUDI) at a flow rate of 30 L/min. Each stage of the MOUDI represented a different particle size range, from 0.05-18 µm. The aerosol particle sizes for each e-liquid were determined gravimetrically by weighing the stages before and after aerosolization. This experiment was performed at 3.9, 4.3, and 4.7 V on the e-cigarette.

The percent of recovered e-liquid was determined for each stage. Stages 8 and 9, representing particle size ranges of 0.31-0.172 µm and 0.172-0.1 µm, consistently collected approximately 90% of the aerosol. Additionally, all 3 voltages produced ultrafine particle sizes, <0.1 µm. Compared to the aerosol of nicotine in an e-cigarette, the e-liquids containing methamphetamine and methadone produced more ultra-fine particles <0.3 µm and had a significantly smaller mean diameter than nicotine. This suggests that the particles of methadone and methamphetamine produced by an e-cigarette are smaller than the particles of nicotine and are can be efficiently deposited into the lung for absorption into the bloodstream.

**Keywords:** Drugs, Particle Size and Distribution, Toxicology  
**Application Code:** Clinical/Toxicology  
**Methodology Code:** Chemical Methods
A promising technology for retrospective monitoring of drug exposure in forensic casework involves measurement of covalent modification of free thiol moieties of blood proteins, such as hemoglobin (Hb), by reactive metabolites (RM) of drugs. Since they typically persist for the life of the protein, such protein “adducts” can provide a much longer window of detection of exposure than is generally possible by direct measurement of parent compound or metabolite. We have previously demonstrated covalent adduction of glutathione and a synthetic thiol peptide in an [i]in vitro[/i] human liver microsome (HLM) based assay by 16 drugs with abuse potential, including methamphetamine, morphine, diazepam, [delta][sup]9[/sup][THC][/sup], by means of LC-QqQ-MS and LC-QTOF-MS. Current work involves assessment of covalent modification of the reactive [sup]93[/sup]Cys thiol of human [beta]Hb by the same drugs as part of the development of a validated assay for retrospective drug exposure. The [i]in vitro[/i] assay procedure was modified to facilitate recovery of adducted Hb by utilizing a dialysis membrane to maintain separation of Hb from microsomal components, while allowing for RM to pass through. For the metabolism/adduction assay, each drug was added to a plastic microfuge tube with residual solvent removed via vacuum centrifuge. HLM were added to the tube and combined with NADPH in sodium phosphate buffer (pH 7.4). Hb was then added and tube incubated at 37[degree]C for 18 h and then centrifuged. Analysis of modified protein was performed using positive ESI on an Agilent 6530 QTOF-MS. Data were collected using full MS scan mode to allow for necessary analysis of all protein components. Both whole protein and tryptic peptide analyses to detect modified Hb were conducted. Preliminary results confirm the formation of adducted protein by reactive abused drug metabolites in the [i]in vitro[/i] system and provide additional proof of concept for continued development of the assay.

Keywords: Clinical/Toxicology, Drugs, Liquid Chromatography/Mass Spectroscopy, Proteomics
Application Code: Clinical/Toxicology
Methodology Code: Liquid Chromatography/Mass Spectrometry
On-site detection methods are very important for the screening of drugs of abuse. Chemical spot tests suffer from frequent false-positives and false-negatives, while immunoassays are expensive and have short shelf-lives. These problems can be overcome using aptamers, oligonucleotide-based bio-affinity elements, isolated in vitro via SELEX. Recently, our lab has made several successful attempts to address these problems for aptamer-based on-site drug detection. We first engineered a new cocaine-binding aptamer that exhibited higher target binding affinity than the originally isolated cocaine-binding aptamer. Using this aptamer, we developed a label-free fluorescence assay based on target-dye displacement for sensitive cocaine detection, with a detection limit as low as 200 nM within seconds. We further discovered the specific inhibition of Exo III by the formation of a target-aptamer complex, and employed this feature to develop a label-free fluorescence assay. To achieve ultra-sensitive detection of drugs in biofluids, we developed a general approach to engineer cooperative-binding split aptamers (CBSAs) that exhibit highly responsive target-induced aptamer assembly for signal reporting. We employed the CBSA to achieve specific, sensitive, one-step fluorescence detection of cocaine within 15 min at concentrations as low as 50 nM in 10% saliva. Additionally, we developed an instrument-free colorimetric assay employing enzyme-assisted-target-recycling-mediated aggregation of CBSA-modified gold nanoparticles for visual detection of low micromolar cocaine within 15 min. Recently, we have adopted our sensor designs into aptamer isolation and have generated new aptamers that bind to a class of designer drugs. The isolated aptamer can be directly adopted into our dye-displacement or CBSA-based sensing platforms. We believe by integrating aptamer isolation with sensor design, robust aptamer-based assays can be generated rapidly for on-site detection of any drug of interest.

Keywords: Bioanalytical, Biosensors, Drugs, Nucleic Acids
Application Code: Bioanalytical
Methodology Code: New Method
There is a need for developing simpler screening methods for drugs in post-mortem samples. Paper spray mass spectrometry is a direct or ambient ionization method in which dried biofluids spots are analyzed directly from paper. Analysis is carried out on dried blood spots directly without sample preparation; total analysis time is about 2 minutes. We have developed a drug screening method for about 140 of the most commonly encountered drugs and drug metabolites in forensic toxicology. In this presentation, we will present the figures of merit, including detection limits, linearity, and selectivity.

Whole blood samples were analyzed by first spotting them on chromatography paper contained within a disposable plastic cartridge. A variety of extraction solvents were investigated; the final solvent was 85-10-5-0.01 acetonitrile-acetone-water-acetic acid. Analysis was carried out using an automated paper spray front-end interfaced with a Q-Exactive Focus in MS/MS mode using an inclusion list. Detection of the drugs was based off of the presence of typically two fragment ions (the quantifier and qualifier) within a 5 ppm m/z window. Fragment ion ratios can be used to decrease the potential for interferences; we elected not to use ion ratios to minimize the chance of false negatives.

An important contribution of this work is the application of paper spray MS to actual post-mortem samples. We analyzed 30 post-mortem blood samples and compared paper spray MS/MS results against an independent HPLC-MS/MS confirmatory assay. Paper spray showed good sensitivity and selectivity compared with the HPLC-MS/MS assay; the true positive rate of paper spray MS/MS was 92.8% and the true negative rate was over 99%. The results indicate that this approach has good potential as a rapid drug screening method in post-mortem toxicology.

**Keywords:** Clinical/Toxicology, Forensics

**Application Code:** Clinical/Toxicology

**Methodology Code:** Mass Spectrometry
The characterization of semiconductor grade germane is challenging not only due to the number of impurities to be determined, but also the differing concentration ranges and types of analytes to be quantitated. The use of a single technology to observe all impurities is not practical due to matrix effects (germane), lack of sensitivity of the instrumentation or the applicability of those techniques to certain analytes. Strategies developed for gas chromatographic detection using mass spectrometry (GC-MS, GC-ICP-MS and GC-TOF-MS) or flame ionization (GC-FID) and other techniques will be reviewed. The applicability of those instrumental techniques to specific impurities in germane and mixtures of germane will be discussed and a cross calibration strategy will be presented for analytes which reference material is not commercially available.

Abstract Text

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Keywords: Gas Chromatography/Mass Spectrometry, GC Detectors, Plasma Emission (ICP/MIP/DCP/etc.), Time
Application Code: Quality/QA/QC
Methodology Code: Gas Chromatography/Mass Spectrometry
Diborane is used in various processes in the semiconductor industry, for the doping of borophosphosilicate glass in DRAM memory devices, deposition of SiB or SiGeB layers in advanced logic devices and as a reducing agent for the deposition of tungsten films in 3D-NAND. Due to the demands for more accurate and precise gas mixtures, the presence of higher borane components in diborane mixtures has become a concern and hence their accurate measurement has become important. In this paper, we present two approaches: FTIR and GC-ICP-MS for this application, and discuss advantages and disadvantages of the two techniques for quantitation. Since gas standards for molecules such as tetraborane, pentaborane, are virtually unavailable, calibration of instrumentation becomes challenging. GC-ICP-MS has advantages for making these measurements because ICP-MS, when employed as a chromatographic detector, has a response that is proportional to the number of atoms in a given molecule. Therefore diborane gas standards that are widely available can be employed as calibrants for tetraborane and pentaborane impurities, provided the number of boron atoms in the standard and unknown are accounted for. This approach has enabled the accurate measurement of higher borane impurity concentrations in diborane mixtures.

Keywords: FTIR, Gas Chromatography, ICP-MS, Specialty Gas Analysis
Application Code: Other
Methodology Code: Gas Chromatography
Fluorinated gases, including CxFy, SF6 and NF3, are critical to diverse applications, from refrigeration to electronics manufacturing. With increasing demand for smart devices and the burgeoning Internet of Things, the global market for these specialty gases is expected to continue to soar over the next several years.

Take nitrogen fluoride (NF3) for example: it is key in the manufacturing of semiconductor chips, flat panel displays and solar cells, where it is employed to clean the process chambers for the manufacturing of silicon chips. Its prevalence today is due to numerous benefits of using NF3 compared to HF and other cleaning agents, which include safety, stability at room temperature, ease of handling, and lower greenhouse gas emissions compared to its predecessors.

The fluorinated specialty gases commonly used throughout the semiconductor process require extremely high purity, demanding an impurity measurement technology that is accurate and sensitive, yet material compatible.

Continuous-Wave Cavity Ring-Down Spectroscopy (CW-CRDS) is a field-proven technique for measurement of a variety of analytes in a suite of gas matrices, ranging from inert to specialty gases. CW-CRDS derives the analyte concentration from monitoring light decay inside a high-finesse optical cavity caused by direct optical absorption of the target molecule. Providing excellent sensitivity and selectivity, CW-CRDS can be applied to even the most challenging applications, such as moisture analysis in gases that are corrosive and spectrally interfering. We present results from the development of CW-CRDS analyzers for detection of trace moisture and hydrogen fluoride impurities in NF3 and other fluorinated gases, with detection limits at single-digit parts-per-billion level.

Keywords: Molecular Spectroscopy, Specialty Gas Analysis, Spectroscopy, Trace Analysis
Application Code: Quality/QA/QC
Methodology Code: Molecular Spectroscopy

Organized Contributed Sessions

Session # 1750  Abstract # 1750-3

Abstract Text
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Keywords: Molecular Spectroscopy, Specialty Gas Analysis, Spectroscopy, Trace Analysis
Application Code: Quality/QA/QC
Methodology Code: Molecular Spectroscopy
In an effort to improve the speed and performance of online GC systems used for the monitoring of permanent gasses and VOCs thru C12; APIX analytics has developed a small system designed around the rugged, handheld, plug-and-play APIX module which contains the entire GC analytical stream. The heart of the module is the proprietary multiphysics detection scheme which couples a highly sensitive microfabricated TCD with the APIX nanoresonator array. The complimentary detector physics allow for increasing sensitivity from ppm level detection of permanent gases thru low ppb level detection of heavier VOCs with increasing sensitivity as analyte mass increases. The system is designed to utilize a parallel analysis structure (up to 4 modules in parallel) with backflush to decrease analysis time. The plug-and-play modules are designed to be interchangeably used with both a 19\" rack mounted format and an ATEX certified design developed in partnership with ASTUTE/EIF for use in potentially explosive environments. Data from both systems will be presented.
An equilibrium isotope fractionation in the liquid-vapor system of CO\(_2\) for C and O isotopes is well established \(i.e\), Grootes et al., 1969, Z. Physik 221]. C isotopes tend to be enriched in \(^{13}\)C in the vapor phase and O isotopes tend to be depleted in \(^{18}\)O. This observation has particular relevance in contemporary stable isotope laboratory practices due mainly to the advent of Continuous Flow-Isotope Ratio Mass Spectrometry (CF-IRMS). For \(^{13}\)C and \(^{18}\)O measurements, CF-IRMS relies almost exclusively on incorporating a high pressure cylinder of CO\(_2\) as a calibrated internal reference gas. If this reference gas contains a liquid phase, the laboratory's ability to produce reliable isotope data will be dependent on whether the isotopic composition of the CO\(_2\) changes during cylinder depletion. Intuitively, one may presume that as the liquid CO\(_2\) within that cylinder decreases, the vapor produced from that liquid will change isotopically to reflect known isotopic fractionation between those phases. This work quantifies the isotopic effect for \(^{13}\)C and \(^{18}\)O as a function of CO\(_2\) cylinder depletion. CO\(_2\) vapor samples from a cylinder that contains both liquid and vapor phases will be taken regularly and measured for both \(^{13}\)C and \(^{18}\)O. CO\(_2\) will be depleted during sequential sampling and resulting cylinder contents monitored gravimetrically. Observed isotopic effects (fractionation) of the vapor from the depleted CO\(_2\) cylinder will be reflected in the \(^{13}\)C and \(^{18}\)O composition of that vapor. Thus the last remaining liquid within the cylinder will likely show the largest isotopic effects in both \(^{13}\)C and \(^{18}\)O in the CO\(_2\) liquid-vapor system. These data will be useful for CF-IRMS researchers that rely on high pressure cylinders for CO\(_2\) reference gas.

Keywords: Isotope Ratio MS, Reference Material
Application Code: Validation
Methodology Code: Mass Spectrometry
The ability to accurately monitor low levels of trace impurities in bulk gases is critical to ensure gas quality and safety. FTIR instruments can measure many components at very low detection limits, and are widely used for impurity analysis in IR non-absorbing as well as IR-absorbing bulk gas. When a smaller number of impurities need to be measured (e.g. CO, CO2, N2O and hydrocarbons in IR non-absorbing bulk gas), new, low-cost Tunable Filter Spectrometry (TFS) technology can be used and achieve even lower detection limits than FTIR. This paper will present data comparing FTIR and TFS performance and capabilities, and will show recent applications of both technologies.

Keywords: FTIR, Molecular Spectroscopy, Process Monitoring, Specialty Gas Analysis
Application Code: Process Analytical Chemistry
Methodology Code: Vibrational Spectroscopy
New gaseous measurement standards can only be offered to society after a lengthy and complex process. The first question national metrology institutes (NMIs) have to ask themselves is “what will be the demands from society in 5 – 7 years from now with respect to new measurement standards and level of accuracy?” Not an easy question at all! If these questions are answered, the research that leads to the development of standard gas mixtures is covering numerous activities. These include thorough purity analysis of the parent gases, test of inner coating of cylinders, search for inert materials for valves and transfer lines and development of high accurate analytical methods to conduct the verification of the prepared standards. When the research is successful, the new gas mixtures have to be tested for their stability. At the same time, participation to comparisons between NMIs gives the confidence (and lower the measurement uncertainty) in the new mixtures. If all of these steps have a positive outcome “the new services passed their halfway point”. Still remaining issues are e.g. writing quality procedures, formulating new CMCs (calibration and measurement capabilities) and having them accepted by other NMIs, bringing the new services under accreditation...and – almost forgotten – making PR about the new services (lectures, newsletters, publications, flyers, revising documentary standards, and so on). During the lecture several of these steps will be highlighted, including challenges encountered, solutions found, current status (e.g. of some passivation treatments and measurement uncertainty sources), as well as for new components, new matrices as for the development of extended gas mixture concentration ranges.

Keywords: Specialty Gas Analysis, Standards, Validation
Application Code: Quality/QA/QC
Methodology Code: New Method
Today's production processes are increasingly under pressure to be competitive in terms of cost and efficiency. The goal of a “lean” production chain is to be robust and efficient, while consistently delivering high product quality at the lowest possible cost. Process analysis is one of the key enablers to achieve this goal.

There are different types of analytical process applications that can accompany a process or product lifespan. At the initial phase, during process development where the target is to define and understand the process design and determine the most efficient and robust operating conditions as well as during scale-up to verify scalability. Within commercial manufacturing, process analysis is also required for process monitoring and real time control.

Testing and evaluating promising and innovative analytical technologies within all three stages is critical. For synthesis gas production and products thereof, there has been a focus on certain spectroscopic techniques for real time measurements within the various process streams. Raman spectroscopy is particularly well suited to analyze synthesis gas components, such as H2, N2, O2, CH4, CO, CO2 etc. for real time applications, which makes it very interesting for the use in monitoring and control of gaseous streams in steam methane reformer technology of natural gas feedstocks to produce hydrogen and carbon monoxide.

Raman spectroscopy offers a rich variety of sampling options which enable multiple remote measurement points with varying temperature, pressure and matrix constituents to be collected simultaneously with one instrument.

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

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

**Methodology Code:** Process Analytical Techniques
Besides its importance as a metabolite in the cellular energy cycle, adenosine triphosphate (ATP) is also a known neuromodulator. A large variety of cells express purinergic receptors in order to respond to extracellular ATP and large dense core vesicles (LDCV) are known for carrying and releasing ATP along with primary transmitters and proteins. Given the many roles of ATP in cellular signaling, it was investigated as a potential candidate for regulation of exocytosis. Our group has previously developed electrochemical techniques for the detection of total vesicular content, both in situ and ex situ. By combining these techniques with the established method of single cell amperometry for detecting released neurotransmitters, the fraction of vesicular content can be determined and the processes of release and storage of neurotransmitters can be picked apart and investigated separately.

Chromaffin cells were incubated for 30 min with ATP and were observed to have an increasingly larger fraction of their vesicular content released during exocytosis. This effect was concentration dependent and also caused faster dynamics of individual release events. Further, incubation with ATP and a catecholamine (norepinephrine) was observed to increase the vesicular content but the fraction released was maintained fairly constant. This effect was also present when incubating isolated vesicles and was not observed with incubation of norepinephrine alone. Pharmacology was used in an attempt to pinpoint the mechanism of action for the effects of ATP on exocytosis. Although the pathway remains undefined and is under continued investigation, these experiments suggest there are still unknown roles for ATP in cellular signaling.

Keywords: Bioanalytical, Biological Samples, Electrochemistry, Neurochemistry
Application Code: Bioanalytical
Methodology Code: Electrochemistry
Because of their low polarity and polarizability, fluorous sensing membranes are both hydrophobic and lipophobic and exhibit very high ion selectivities. Here we report a new fluorous-phase ion-selective electrode (ISE) with a wide sensing range centered around pH 7, suitable for physiological pH sensing. The new fluorophilic ionophore tris[perfluoro(octyl)butyl]amine was synthesized and tested with a newly designed electrode body with improved mechanical sealing that offers much improved measurement reliability. In a 1 M KCl background, the fluorous-phase ISEs based on this new ionophore exhibit a sensing range from pH 2.2 to pH 11.2, which is one of the widest working ranges reported to date. High selectivities against common interfering ions such as K\(^{+}\), Na\(^{+}\), and Ca\(^{2+}\) are determined (Selectivity Coefficients: \(\log iK_{H,K}^{\text{pot}} = -11.6\); \(\log iK_{H,Na}^{\text{pot}} = -12.4\); \(\log iK_{H,Ca}^{\text{pot}} < -10.2\)).

**Abstract Text**

Because of their low polarity and polarizability, fluorous sensing membranes are both hydrophobic and lipophobic and exhibit very high ion selectivities. Here we report a new fluorous-phase ion-selective electrode (ISE) with a wide sensing range centered around pH 7, suitable for physiological pH sensing. The new fluorophilic ionophore tris[perfluoro(octyl)butyl]amine was synthesized and tested with a newly designed electrode body with improved mechanical sealing that offers much improved measurement reliability. In a 1 M KCl background, the fluorous-phase ISEs based on this new ionophore exhibit a sensing range from pH 2.2 to pH 11.2, which is one of the widest working ranges reported to date. High selectivities against common interfering ions such as K\(^{+}\), Na\(^{+}\), and Ca\(^{2+}\) are determined (Selectivity Coefficients: \(\log iK_{H,K}^{\text{pot}} = -11.6\); \(\log iK_{H,Na}^{\text{pot}} = -12.4\); \(\log iK_{H,Ca}^{\text{pot}} < -10.2\)).

**Keywords:** Bioanalytical, Environmental/Biological Samples, Ion Selective Electrodes, Potentiometry

**Application Code:** Bioanalytical

**Methodology Code:** Electrochemistry
Conducting polymers possess various very unique physical properties that can be applied for many different applications. Some variations in physical properties of conducting polymers are suitable for analytical and bioanalytical purposes, e.g. some conducting polymers can be used as matrixes for the immobilization of biomolecules and in such way they can be applied in the design of electrochemical biosensors [1], some – can be applied as charge transfer matrixes [2], some –can be imprinted by molecular imprints of molecules and applied in chemical sensors for large and low mass analytes [3]. In previous our research we have reported that electrochromic properties of conducting polymer, polyaniline, can be applied in the determination of some heavy metals [4]. Therefore applicability of electrochromic properties of conducting polymer, polyaniline, in sensor design will be the main topic of this presentation. In recent our research electrochromic sensitivity of polyaniline towards pH changes was observed and evaluated. Potential-dependent spectral properties of polyaniline will be shown and discussed. Development of electrochromic pH sensors based on conducting polymer, polyaniline, which is electrochemically deposited on Indium Tin oxide covered glass will be presented. Applicability of such electrochromic structures in the design NH3 and NH4+ sensing systems will be discussed.

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References
Bioanalytical - Electrochemistry

Spectroelectrochemical Cross-Correlation During Redox Cycling in Zero-Dimensional Nanopore Electrode Arrays

The ability of zero-mode waveguides (ZMW) to guide light into subwavelength-diameter nanoapertures has been exploited for studying electron transfer dynamics in zeptoliter-volume nanopores under single-molecule occupancy conditions. Arrays of nanopore-confined recessed dual ring electrodes fabricated using layer-by-layer deposition coupled with focused ion beam etching can function both as working generator-collector electrode pairs and also as ZMW arrays. We have articulated these structures with an optically transparent bottom, so that the nanopores are bifunctional, exhibiting both nanophotonic and nanoelectrochemical behaviors allowing the coupling between electron transfer and fluorescence dynamics to be studied under redox cycling conditions. Flavin mononucleotide, FMN, contains an isoalloxazine chromophore which is fluorescent in the oxidized state, while the reduced state, FMNH$_2$, exhibits a substantially lower quantum efficiency, thus permitting the redox state of single FMN molecules to be followed by observing their fluorescence behavior. Because the ~100 zeptoliter volumes of these nanopores dictate very short residence times, evidence for single molecule redox cycling is obtained from the fluorescence dynamics. Freely diffusing species exhibit characteristic behavior in which the probability of observing single reduced molecules increases as the potential is scanned to more negative values. Conversely, single molecule cycling behavior is evidenced by the distribution of on- and off-times, which are altered relative to freely diffusing FMN/FMNH$_2$. Proof-of-principle experiments are conducted showing that electrochemical and fluorescence signals may be correlated to reveal single molecule fluctuations in the array population. Cross-correlation of single molecule fluctuations in amperometric response and single photon emission provides unequivocal evidence of single molecule sensitivity.

Abstract Title
Spectroelectrochemical Cross-Correlation During Redox Cycling in Zero-Dimensional Nanopore Electrode Arrays

Primary Author
Donghoon Han
University of Notre Dame

Co-Author(s)
Garrison M. Crouch, Kaiyu Fu, Paul Bohn

Abstract Text
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Keywords: Bioanalytical, Spectroelectrochemistry
Application Code: Bioanalytical
Methodology Code: Electrochemistry
Fluorescence/Luminescence

Exploring the Multidimensionality of Luminescence Spectroscopy to Face Current Analytical Challenges

Andres Campiglia
University of Central Florida

Date: Thursday, March 1, 2018 - Morning
Time: 08:30 AM
Room: 308D

Abstract Text

The multidimensional nature of photoluminescence phenomena provides fluorescence and phosphorescence techniques with unique potential for the analysis of complex matrices. The simplicity of experimental procedures makes room-temperature luminescence techniques the most popular approach. The main limitation of room-temperature techniques is the broad nature of excitation and emission spectra. Reducing the sample temperature offers several advantages. Luminescence quantum yields often increase, and the complications of oxygen quenching and energy transfer are eliminated. Temperature effects on luminescence are specially pronounced in the so-called high-resolution photoluminescence techniques. In these low-temperature techniques, sharp spectra with vibrational information result from homogeneous and in-homogeneous band-broadening reduction.

This presentation focuses on significant improvements we have made to line-narrowing luminescence techniques. The complications of traditional low-temperature methodology are eliminated by using a cryogenic fiber optic probe with the distal end frozen directly into the sample matrix. We can now routinely perform measurements at liquid nitrogen (77K) and helium (4.2K) temperatures; frozen samples are prepared in a matter of seconds. The full dimensionality of photoluminescence phenomena is obtained with an instrumental system capable to record wavelength time matrices (WTMs) and time-resolved excitation-emission matrices (TREEMs) in short analysis time. The application of these data formats to the analysis of polycyclic aromatic compounds released into the Gulf of Mexico by the Deepwater Horizon event will be discussed.

Acknowledgements: The authors acknowledge financial support from The Gulf of Mexico Research Initiative (Grant 231617-00). The views expressed are those of the authors and do not necessarily reflect the view of this organization.

Keywords: Environmental Analysis, Fluorescence, Luminescence, PAH
Application Code: Environmental
Methodology Code: Fluorescence/Luminescence
Phospholipid vesicles have been extensively utilized as bioreactor and biosensor platforms for intracellular applications. The preparation of biosensors requires sensing components, including enzymes or proteins, and non-destructive approaches to stabilize the sensor platform. Phospholipid vesicles can protect the encapsulated species but the inherent impermeability of bilayer inhibits transport of ions or small biomolecules towards the vesicle interior. A porous, stabilized phospholipid nanoshell (PPN) containing fluorescent protein was prepared for specific and selective detection of glucose. The PPN sensor was prepared using polymerizable phospholipid bis-SorbPC encapsulating the fluorescent protein (FRET sensor protein, FLIPglu-600µ) inside the aqueous core of PPN. Upon binding of substrate to the FLIPglu-600µ sensor showed a concentration-dependent change in fluorescence resonance energy transfer between the attached chromophores related to the binding affinity of glucose to sensor proteins. The stability and porosity of the bis-SorbPC vesicles were adjusted by forming polymer networks within the bilayer. The preparation of the polymer scaffold was optimized by varying the lipid/monomer to initiator ratio and varying the polymerization conditions, with the aim of retaining sensor function. The stabilized porous liposomes loaded with fluorescent protein allowed size-selective diffusion of glucose and other small biomolecules into the core of the PPN to interact with encapsulated sensor proteins. This unique combination of stability and porosity enables a new paradigm for intracellular measurements of small analytes.
Metal ions are widespread in nature, of essence of many biological processes and industrial relevance; yet their presence or absence beyond requisite concentration threshold impart lethal toxic effect to the living systems/organisms. Therefore, their on-site, real time selective detection at requisite concentration is highly indispensable for physiological necessities and environmental impact assessment of contaminants. Molecular systems those exhibit quantifiable signal transduction, particularly luminescence perturbation, upon analyte binding offer strategic and operational advantages, where their synthetic perspective are driven by various methodological designs, modulation of involved photo-physical processes and manifestation of output luminescent/colourant signal. Since Czarnik’s report (1997), advances in xanthene dye derivative based colourant and fluorescent systems for detection of various analytes owes not only to their signal modulation in aqueous media, but also for their excellent spectroscopic properties and contrast structure-function correlation. We have established a rationality in development of ‘amino-alkyl-rhodamine’ based systems through structural and functional modification for selective and sensitive detection of few targeted metal ions with fluorescence and absorption spectral modulation and/or colourant methods. On their immobilization onto an inorganic solid surface, such rhodamine derivatives can capture and remove metal ions from the solution. The potential and scope of these molecular entities are extended with fluorescence imaging in detection and concentration level monitoring of bio-accumulated toxic metal ions in root/shoot tissues of various plants as they uptake essential and non-essential metal ions along with other nutrients. The presentation will focus on significant parameters that will induce and influence their rational development for selective detection.
Developing methods that can be used to quickly, easily, and reliably detect low concentrations of small molecules in a cost effective manner has long been a priority for researchers because these methods play an important role in biomedicine, environmental analysis, and many other fields. Since traditional chemical-physical methods are expensive and cumbersome to perform, many alternatives have been explored, including biotechnology. Although there have been many examples of biosensors for a myriad of compounds and applications, they often have significant limitations. This is especially true of the cell- and protein-based sensors. Cell-based sensors often lack sensitivity while protein-based sensors generally require complex steps for preparation and use. In this work, we present a novel biosensor that exploits the morphology of bacterial endospores to create spores engineered to be analytical tools (SEATs) in an attempt to address the limitations of more traditional biosensors. To demonstrate this concept we have developed SEATs designed to identify arsenic, a toxic carcinogen that was identified by the ATSDR as the single most concerning hazardous compound in 2015. This was accomplished via the expression of a tripartite fusion protein that combines the B. subtilis spore coat protein CotB, the arsenite binding protein ArsR, and the fluorescent reporter EGFP N-terminus to C-terminus, respectively, on the surface of Bacillus megaterium spores. These SEATs have limits of detection as low as 1 x 10\(^{-11}\) M, lower than most traditional cell-based techniques and well below the guidelines for arsenic contamination set by the EPA. SEATs also retain the low cost and ease of production and use of cell-based assays, unlike protein-based sensors. Due to their sensitivity, speed, and low cost, we believe that SEATs have great potential for use in many biomedical and environmental applications.
Investigating the diffusion of nanoparticles on lipid bilayer is important in understanding many biological processes on cell membrane. Molecules and nanoparticles are observed to diffuse laterally on lipid bilayers. Conventionally, this diffusion is believed to be confined in the two-dimension (2D) plane, i.e., 2D planar diffusion. Recently, evidence shows that desorption of the nano-objects may be involved in the process and play an important role in their lateral movement, i.e., desorption assisted diffusion. With conventional 2D single particle tracking (SPT) techniques, the diffusion detail in z direction is missing thus it is challenging to differentiate these different diffusion modes. By using our 3D SPT technique, we successfully recovered the particles’ diffusion on lipid bilayer in the whole 3D space. Different diffusion modes under various conditions were observed. At physiological pHs, both unmodified and TAT-modified nanoparticles showed 2D planar diffusion on synthetic lipid membrane. At pH 10, the particles showed confined diffusion within “domains”, and “hopping” between different domains. According to our observation, the hopping can involve two different modes: the in-plane mode during which the particle stayed within the lipid layer, and the out-of-plane modes, during which the particle desorbed from the lipid bilayer and moved in the solution before it landed on the lipid bilayer again. These observations were not made using 2D SPT in the past and will help us understand how particle and lipid bilayer interact with each other.
The effect of ligand on the lateral diffusion of receptor for advanced glycation end products (RAGE), a receptor involved in numerous pathological conditions, remains unknown. Single particle tracking experiments that use quantum dots specifically bound to hemagglutinin (HA)-tagged RAGE (HA-RAGE) are reported to elucidate the effect of ligand binding on HA-RAGE diffusion in GM07373 cell membranes. The ligand used in these studies is methylglyoxal modified-bovine serum albumin (MGO-BSA) containing advanced glycation end products modifications. The binding affinity between soluble RAGE and MGO-BSA increases by 1.8 to 9.7-fold as the percent primary amine modification increases from 24 to 74% and with increasing negative charge on the MGO-BSA. Ligand incubation affects the HA-RAGE diffusion coefficient, the radius of confinement, and duration of confinement. There is, however, no correlation between MGO-BSA ligand binding affinity with soluble RAGE and the extent of the changes in HA-RAGE lateral diffusion. The ligand induced changes to HA-RAGE lateral diffusion do not occur when cholesterol is depleted from the cell membrane, indicating the mechanism for ligand-induced changes to HA-RAGE diffusion is cholesterol dependent. The results presented here serve as a first step in unraveling how ligand influences RAGE lateral diffusion.

**Keywords:** Bioanalytical, Fluorescence

**Application Code:** Bioanalytical

**Methodology Code:** Fluorescence/Luminescence
Neurotransmitters manage a wide range of cognitive functions, which include motivation, behavior, learning, and memory. The amount of dopamine is an important factor in various diseases, and is one of the markers used in the diagnosis of several conditions. Moreover, oxytocin is involved in social affiliation, and dysregulation of oxytocin levels causes autism and other developmental disorders associated with increased anxiety and deficit in social behavior. Consequently, there is a strong need to develop efficient and rapid methods that can be used to selectively determine and continuous changes in the dopamine and oxytocin levels.

We report the developments of fluorescent dopamine (compound [b]1[/b]) and oxytocin (compound [b]2[/b]) sensors. Compound [b]1[/b] was constructed using the cyanopyranyl group as the fluorophore and a Fe\([\text{II}]\) complex both as the ligand exchange site and fluorescence quenching moiety. In contrast to the weak fluorescence emission of [b]1[/b] in the absence of dopamine, a much stronger fluorescence emission was observed following the addition of dopamine owing to the release of Fe\([\text{II}]\) from [b]1[/b]. The reaction of [b]1[/b] with dopamine was not affected by the presence of foreign substances.

Compound [b]2[/b] was constructed from the fluorophore and a peptide as the binding site of oxytocin. Compound [b]2[/b] indicated a much stronger fluorescence emission when associated with oxytocin, and the binding and detection of [b]2[/b] with oxytocin was not influenced by the presence of foreign substances.

The experimental results clearly showed that these compounds are good fluorescent indicators, and can be widely employed in neurotransmitter detection protocols.

Keywords: Bioanalytical, Biosensors, Fluorescence, Spectrophotometry
Application Code: Bioanalytical
Methodology Code: Fluorescence/Luminescence
A cannabis edible, also called cannabis-infused food, is a food product that contains cannabinoids, especially THC and CBD. Cannabis edibles are consumed for both medical and recreational purposes. Because cannabinoids are soluble in lipids and alcohols, cannabis must be cooked with one of these two substances in order to infuse the cannabinoids into the food. The oil-solubility of cannabis extracts has been known since ancient times. Since the infused cannabinoids are a concentrate it is important to monitor chemical residues in the concentrate, specifically pesticides. However, it can be difficult to analyze for pesticide residues in high lipid content matrix. Lipids can cause both analysis and instrument issues over time. QuEChERS and organic extractions are commonly used sample preparation methods for the analysis of pesticides from food products however the dispersive SPE containing C18 which is used for fatty matrix is insufficient at selectively removing lipids. A novel sorbent Enhanced Matrix Removal-Lipid (EMR-Lipid) specific for the removal of lipids has shown to be very effective at retaining lipids without compromising recovery of the pesticides. The application will show the matrix removal capabilities of newly formulated EMR-Lipid specific for high lipid content cannabis-infused food products and the analysis of pesticides.

Keywords: Food Contaminants, Liquid Chromatography/Mass Spectroscopy, Pesticides, Sample Preparation
Application Code: Food Safety
Methodology Code: Sampling and Sample Preparation
Food Safety - Sensors, GC/MS, and Others

High Throughput Residual Analysis of Pesticides and Environmental Contaminants in Poultry and Meats

Tolerances, or maximum residue limits (MRLs) are established for contaminants to comply with food trade regulations and to protect consumers' health. Efficient, high throughput and sensitive methods for analysis of multiple classes of contaminants are needed to provide fast turnaround time while keeping testing cost reasonably low. We developed and validated a fast, simple, high throughput analytical method for simultaneous determination of 270 organic contaminants, including environmental contaminants: PAHs, PCBs PBDEs and other flame retardants, and diverse pesticides in poultry and meat muscle. Sample preparation was based on QuEChERS extraction with acetonitrile. The extracts were split, with one aliquot analyzed by UHPLC-MS/MS for 100 LC-amenable pesticides, and another aliquot subjected to automated SPE mini-column cleanup using Instrument Top Sample Preparation (ITSP), followed by low pressure (LP)GC-MS/MS analysis of 210 GC-amenable analytes. Both instrumental analysis run were 10 min, and 50 pesticides were analyzed by both (LC- and GC-) techniques, providing an additional degree of confirmation. The method was validated in poultry (chicken) and meats (cattle and pork) muscle tissue at ½, 1 and 2X of the USA tolerance (X) levels. Using isotopically-labeled internal standards, satisfactory recoveries (70-120%) with RSDs ≤20% were achieved for ~90% of the tested contaminants. The developed method is fast and cost-effective, with calculated sample preparation cost $6 per sample. Method detection limits were 1-5 ng/g for most contaminants, allowing their accurate measurements below tolerance levels.

Keywords: Food Contaminants, Gas Chromatography/Mass Spectrometry, Liquid Chromatography/Mass Spectrometry
Application Code: Food Safety
Methodology Code: Sampling and Sample Preparation
Evaluation of a High Throughput, No DCM or Capital Equipment Sample Clean Up for POPs Analysis

The continued interest in Persistent Organic Pollutants (POPs), such as polychlorinated dibenzo-p-dioxins (PCDDs), furans (PCDFs), biphenyls (PCBs) and PBDEs, has led to a variety of attempts to automate both the extraction and cleanup of complex sample matrices. This has resulted in development of automated Pressurized Liquid Extraction (PLE) as an alternative to Soxhlet, and of fully automated sample cleanup instead of manual preparative open column chromatography.

In this contribution, we have developed a semi-automated system which is cheap and effective for fast and reliable sample clean up, using a relatively simple approach. An important feature of this technique is that a minimum amount of solvent is used, and no dichloromethane is used at all. This is important since many laboratories around the world are phasing out this solvent.

The semi-automated system is composed of a laboratory funnel, filled with hexane or toluene, a solvent pump and three pre-packaged columns: high capacity acid-base-neutral silica (lower capacity possible), carbon and alumina. The sample can be injected directly on top of the silica column. The sample is eluted through the silica column onto carbon and alumina and the analytes are eluted from both columns with toluene into separate fractions. Total solvent use depends on the lipid capacity of the silica used and varies between 100 - 250 mLs. Prepackaged column kits for Dioxin & PCB analysis reduce risk of background contamination. Total processing time for sample cleanup is less than 45 minutes. An unlimited number of parallel sample cleanup channels can be used.

A variety of matrices, such as eggs, feed, and oils, showed very good recoveries (70%-110%) for both PCDD/Fs and PCBs. Because the system is mostly composed of disposable parts, the risk of cross-contamination is very low. The system can be set up at low cost. The certified columns and simple semi-automated system guarantee same morning or afternoon POPs analysis.

Keywords: Food Safety, Gas Chromatography/Mass Spectrometry, PCB's, Trace Analysis
Application Code: Food Safety
Methodology Code: Sampling and Sample Preparation
We have developed the Surface Plasmon Resonance (SPR) immunosensor for a practical application in small molecule detection. In order to detect a small analyte, the indirect competitive inhibition immunoassay with secondary immunoreaction was employed. In our past research, the sensitivity of 150 ppq (fg mL⁻¹) was achieved in PBS buffer solution. However, our SPR immunosensor did not respond to the monoclonal antibody of beta agonist in urine sample. Therefore, the protocol for a real sample analysis is studied here.

Immunoassay is well-known as a highly selective sensing protocol. However, an immunoassay often faces a challenge in a reproducibility. To date, it was inferred that it may be caused by handling error. At beginning of this work, the reason of handling error of urine sample was studied in this research. As a result, it was noticed that pH of real urine highly affected on the immunoreaction. Therefore, pH was adjusted by mixing with PBS buffer solution (1:1). The pH of sample solution became always 7.4 by the buffer effect. However, SPR did not show any response of immunoreaction.

Next, it was examined the removal of inhibitors from urine sample. Here, the filtration methods for urine of cows were studied. It was found that the filtration using pore size could not remove inhibitors. But, the inhibitors were totally removed by the filter modified with functional group such as –CH₃, –NH₂, or –COOH. It was noticed that the COOH-modified silica filter showed the best performance. It was almost comparable sensitivity to that in PBS buffer solution. It will be discussed that the mechanism of inhibitors in urine.

In summary, the protocol of the detection of beta agonist, clenbuterol in urine was studied. Clenbuterol is problematic material in food safety. It is proposed that the pretreatment method for the ppq-level detection of clenbuterol in urine sample.

**Keywords:** Biosensors, Food Safety, Sensors

**Application Code:** Food Safety

**Methodology Code:** Sensors
Food Safety - Sensors, GC/MS, and Others

"Not Your Kid’s Apple Juice": An Examination of Arsenic Content in American and European Hard Ciders

Hard cider, or alcoholic cider, is an alcohol fermented beverage produced primarily from apples. Hard ciders have a long history around the world but only have become readily available in the United States over the past two decades. Over the last several years, several studies have been conducted showing the presence of arsenic in apple juices and wine.

The historical and continued use of arsenic based pesticides around the world and the exposure of crops to arsenic from potentially arsenic laden fertilizers can expose agricultural products to arsenic contamination. Arsenic based pesticides, particularly lead arsenate, were in widespread and common use in the United States up until the 1970’s until its final ban in 1988. Despite arsenic residue being recognized as a potential problem from the turn of the century, lead arsenate was one of the most widely used pesticides in the nation and was applied to millions of acres of crops through the 1940’s before being replaced by DDT.

Lead arsenate was the most commonly applied pesticide in apple orchards since it was effective against the codling moth which to this day is one of the most persistent apple harvest pests. Many of the historical apple orchards around the world still produce apples and apple products, and so potential for contamination of arsenic compounds remain. Heavy metal pesticides were designed to be persistent and this issue can cause environmental and health problems decades after the pesticides were banned.

In this study, samples were obtained of popular American and European hard ciders. Modern hard ciders are produced from either fresh apples or apple concentrates. American ciders are required to be at least 50% apples or concentrate, while UK ciders are required to be at least 35% apples. Samples were digested using microwave digestion and testing by ICP-MS and LC-ICP-MS to determine total arsenic content and potential content of different arsenic species.

Keywords: Beverage, Food Safety, ICP-MS, Liquid Chromatography/Mass Spectroscopy
Application Code: Food Safety
Methodology Code: Atomic Spectroscopy/Elemental Analysis
**Determination of Some Heavy Metals in Slippery Elm Bark Samples**

Slippery Elm (Ulmus Rubra) inner bark is used in herbal medicine (both human and veterinary medicine) and the concentration levels of heavy metals in herbal treatments are important because they may influence people using these treatments. Samples of slippery elm bark powder received from three different suppliers were homogenized, digested (by wet acidic digestion) and dissolved. The concentrations of several heavy metals in the above samples were determined by inductively coupled plasma – optical emission spectrometry (ICP-OES) and graphite furnace atomic absorption spectrometry (GF-AAS). Additionally, some samples of slippery elm bark were also examined by ATR-FTIR spectroscopy and Raman spectroscopy. The analytical results will be presented, summarized and compared.

**Keywords:** Analysis, Atomic Spectroscopy, Food Contaminants, Metals

**Application Code:** Food Safety

**Methodology Code:** Atomic Spectroscopy/Elemental Analysis
Polychlorinated dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs) are toxic persistent organic pollutants (POPs) that occur as unwanted byproducts of several industrial and natural processes. The persistence, bioaccumulative nature and high toxicity (particularly of the 2,3,7,8 substituted congeners) of these substances, require that they are carefully monitored to minimize entry into the food chain. Quantification and confirmation of these POPs in food and feedstuffs at the levels stipulated by the European Union (EU) requires sensitive, selective and robust analytical instrumentation.

In this study the performance of a Thermo Scientific triple quadrupole GC-MS/MS was evaluated using both solvent standards and food/feedstuffs samples for the analysis of PCDD/Fs to satisfy regulatory requirements. Suitably set limits of quantification (LOQs) were employed in order to show compliance to 1/5th maximum levels and obtain the sensitivity and robustness required in high throughput routine laboratories. The data presented in this study demonstrates highly sensitive, reproducible and robust GC-MS/MS analysis with highly flexible data reprocessing and reporting features.

Keywords: Gas Chromatography/Mass Spectrometry, Quantitative, Semi-Volatiles, Tandem Mass Spec
Application Code: Food Safety
Methodology Code: Gas Chromatography/Mass Spectrometry
Oxidation of DNA by reactive oxygen species (ROS) yields 8-oxo-7,8-dihydroguanosine (8-oxodG) as the primary oxidation product, which can lead to downstream G to T transversion mutations. DNA mutations are nonrandom, and mutations at specific codons are associated with specific cancers, as widely documented for the p53 tumor suppressor gene. Here, we present the first direct LC-MS/MS study of primary oxidation sites of p53 exon 7. We oxidized a 32 base pair (bp) double-stranded (ds) oligonucleotide representing exon 7 of the p53 gene. Oxidized oligonucleotides were cut by a restriction endonuclease to provide small strands and enable sites and amounts of 8-oxodG to be determined directly by LC-MS/MS. Oxidation sites on the oligonucleotide generated by two oxidants, catechol/Cu\(^{2+}\)/NADPH and Fenton’s reagent, were located and compared. Guanines in codons 243, 244, 245 and 248 were most frequently oxidized by catechol/Cu\(^{2+}\)/NADPH, while Fenton’s reagent oxidations were more specific for guanines in codons 243 and 248. Modeling of docking of oxidation agents on the ds-oligonucleotide were consistent with the experimental oxidation sites. Significantly, codons 244, 248 are mutational “hotspots” in non-small cell and small cell lung cancers, suggesting a possible role of oxidation in p53 mutations leading to lung cancer.
Antibody-drug conjugates (ADC) have become a very promising therapy for cancer treatment because of their capability of delivering highly toxic drugs to cancer cells selectively while remaining the normal cells been affected as less as possible. Though the toxic drugs are conjugated to the antibody via linkers, they are still expected to detach from the antibodies over time after administration, which will lead to serious side effects and change the drug to antibody ratio (DAR), and thus further affect the treatment efficacy. Therefore, it is very crucial to understand the DAR change in plasma during the treatment. The factors such as the large molecular weight, wide mass distribution, and the DAR variation make it very challenging for the DAR detection. In this study, we developed an intact antibody/ADC analysis method to detect ADC in mouse plasma by high resolution LC-MS (AB Sciex QTOF 6600). Briefly, the antibody/ADC in the mouse plasma was first captured by anti-human IgG antibody immobilized on a magnetic beads by biotin-streptavidin interaction, the captured antibody/ADC was washed with buffer followed by Deglycosylation by Rapid PNGase F. The beads with deglycosylated antibody/ADC were isolated and washed with buffer and water prior to elution of antibody/ADC from the beads by 20% ACN in water with presence of 0.2% formic acid. The prepared samples were analyzed by LC-QTOF. The detailed experimental conditions and results will be present at the conference.

This project is supported by Frontage Laboratories, Inc.
Secreted glycoproteins are of great importance to regulate the intercellular communication and to mediate diverse physiological processes. However, it is still a daunting task to systematically analyze secreted glycoproteins because their abundance is low compared to highly abundant proteins in commonly used Fetal Bovine Serum (FBS). In this work, the global and site-specific analysis of N-glycoproteins in human cells was achieved by integrating metabolic labeling, click-chemistry-based enrichment and MS-based proteomics. Secreted N-glycoproteins in MCF7 cells were metabolically labeled with N-azidoacetylgalactosamine (GalNAz). Different methods were compared to enrich labeled glycoproteins. The used alkyne derivatives included DBCO-sulfo-biotin, DBCO-magnetic beads and alkyne-magnetic beads. For DBCO-sulfo-biotin, NeutrAvidin beads were employed to capture biotinylated glycoproteins and subsequently were released by PNGase F. For magnetic beads, digestion on beads was performed after stringent wash. The glycopeptides were eluted by PNGase F and analyzed by LC-MS/MS (LTQ-Orbitrap). Ninety-two glycosylation sites were identified on 59 proteins in the experiment of alkyne-magnetic beads. Meanwhile, 46 glycosylation sites were identified on 32 proteins using DBCO-magnetic beads, while the DBCO-sulfo-biotin enrichment method identified 22 glycosylation sites on 20 proteins. The Gene Ontology clustering of the identified glycoproteins indicates that most of the proteins are located at extracellular region. Protein clustering based on molecular function and biological process shows the proteins possess catalytic activity, and receptor binding, which are closely related with protein extracellular activities. We globally and site-specifically analyzed secreted N-glycoproteins in MCF7 cells by integrating metabolic labeling and click chemistry, which can be extensively used for secreted protein analysis.

This project was supported by NSF (CAREER Award, CHE-1454501).
Accurate and reliable quantification of endogenous lipid mediators in complex biological samples is a daunting challenge. In this study, a robust and direct endogenous quantitative method using background subtracting calibration curves by liquid chromatography-tandem mass spectrometry was firstly developed for the determination of endogenous lipid mediators in ischemic stroke rats. Absolute quantification without surrogate matrix could be achieved by using background subtracting calibration curves, which were corrected and verified from standard curves constructed on original matrix. The recoveries of this method were in the range of 50.3-98.3%, the precision with the relative standard deviation was less than 13.8% and the accuracy with the relative error was within ±15.0%. In addition, background subtracting calibration curves were further verified by validation factors ranged from 90.3% to 110.9%. This validated method has been successfully applied to the analysis of seven endogenous inflammation-related lipid mediators in the brain tissues of ischemic stroke rats. The results indicated that prostaglandins as inflammatory factors and some lipid mediators with neuroprotective effects increased apparently (p < 0.05) in the stroke groups compared with the normal rats. Besides, the two drugs (isosteviol sodium and edaravone) could significantly reduce (p < 0.05) the levels of PGE2 and PGF2\* of stroke rats to inhibit inflammation. Based on the results, it is strongly believed that this approach can be readily generalized as a new reference for the quantification of endogenous compounds in the complex biological samples.
Neurochemicals represent a structurally and functionally diverse group of molecules essential to the function of the nervous system. Central to understanding each neurochemical’s role in various physiological states is the ability to measure concentration dynamics in vivo. Elucidating the complex relationships between different neurochemicals requires targeted assays capable of simultaneously monitoring multiple analytes with high sensitivity and specificity. Liquid chromatography-mass spectrometry (LC-MS) coupled to microdialysis can be implemented to address such analytical challenges. Analytes are separated from their biological matrices using reverse-phase liquid chromatography and coupled directly to ESI-MS/MS for unambiguous identification and quantification. By tailoring the LC-MS assay to the analytes of interest, it is possible to investigate a wide variety of biomolecules across a range of physiological concentrations. For small molecule neurotransmitters and their metabolites, we have developed an assay using benzoyl chloride derivatization to improve reverse-phase retention and ESI sensitivity. Dialysate samples from 1-3 mL can be rapidly analyzed (< 3 minutes) for 23 compounds, yielding physiologically relevant limits of detection for all analytes (ie. sub-nM for monoamines). For another class of neurochemical, neuropeptides, fewer generated samples and lower concentrations demand longer run times and more sensitive methods. Here we demonstrate the use of capillary LC-MS for analysis of a group of neuropeptides known as the melanocortins. Utilizing improved sensitivity afforded by capillary columns and nanoESI flow rates we can achieve limits of detection in the 1-5 pM range at runtimes under 15 minutes. This work represents the fastest implementation of LC-MS using benzoyl chloride derivatization as well as the first quantitative assay of the melanocortin peptides.

This work was supported by NIH Grants # R01DK066604 and R37EB003320

Keywords: Bioanalytical, Capillary LC, Liquid Chromatography/Mass Spectroscopy, Neurochemistry
Application Code: Bioanalytical
Methodology Code: Liquid Chromatography/Mass Spectrometry
A simple, rapid, sensitive, selective and reproducible HPLC-MS/MS method was developed for qualitative and quantitative analysis of all proteinogenic amino acid enantiomers and several non-proteinogenic amino acids. The optimized HPLC-MS/MS method enabled all the chiral amino acids to be enantioseparated by using teicoplanin and quinine based stationary phases. Amino acids were derivatized with 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC) prior to analysis, and they were detected by multiple reaction monitoring in the positive mode, which showed great sensitivity with detection limits down to 0.1 to 5 ng/mL. This analytical platform showed important applications in the sensitive assessment of low abundant D-amino acids in complex biological samples. With the new method, free D-amino acids in the whole brain of mutant ddY/DAO- mice (lacking D-amino acid oxidase activity), and control ddY/DAO+ mice (wild-type) are quantitatively analyzed. The concentrations of L-amino acids are similar in wild-type mice brains compared to the mutant mice brains. D-Leu, D-Pro, D-Ser, D-Ala and D-Arg showed elevated concentration in ddY/DAO- mice.
Over 2.5 million traumatic brain injuries (TBIs) occur each year in the United States and are involved in 30% of all injury-related deaths. Current TBI assessment techniques mainly rely on subjective neuropsychiatric symptoms or advanced neuroimaging techniques to determine whether additional monitoring and treatment are necessary. In addition, the limited sensitivity in assessing mild or delayed symptoms make these techniques unable to definitively exclude TBI. New understanding on the disruptive effects that a traumatic brain injury has on neuronal metabolism has revealed a complex cascade of pathophysiological changes with implications for TBI characterization. In this study, a high-performance liquid chromatography – tandem mass spectrometry (HPLC-MS/MS) method was developed for the separation and detection of ten urinary metabolites previously associated with TBI clinicopathology, including norepinephrine, homovanillate, glutamate, 5-hydroxyindoleacetate, lactate, pyruvate, glycerol, N-acetylaspartate, F2\textsubscript{\textalpha}-isoprostane, and 4-hydroxynonenal. The resulting method features rapid analysis times (<10 min), minimal sample preparation, and the separation of disparate groups of metabolites within a single run. The validated method was shown to possess excellent sensitivity (method detection limits: 0.01 µg/L – 5 µg/L), good reproducibility (RSD: 1-8%), and good accuracy (82-107%). Finally, the method was applied to urine specimens collected from military personnel training at Fort Leonard Wood, Missouri who presented with concussions at the General Leonard Wood Army Community Hospital. The detailed findings of the newly developed method will be presented at the symposium.

Keywords: Bioanalytical, Biomedical, Liquid Chromatography/Mass Spectroscopy, Method Development

Application Code: Biomedical

Methodology Code: Liquid Chromatography/Mass Spectrometry
A novel polymerized bonded phase on silica was designed to minimize or avoid the need for TFA while also increasing chromatographic resolution, as compared with conventional alkysilane bonded phases. Monoclonal antibody (mAb) related drugs represent one of the fastest growing sectors of the pharmaceutical industry. Due to their structural heterogeneity generated during expression, processing and storage, there is a demand for mAb impurity identification by LCMS. Currently, LCMS of mAbs suffers from poor LC resolution and MS sensitivity under the conditions of 0.1% formic acid (FA) plus 0.025% trifluoroacetic acid (TFA). In this paper, different ESI-compatible mobile phase acidic modifiers are compared for use with the polymer bonded phase, including FA and difluoroacetic acid (DFA). RPLC-MS is performed on an intact IgG1 sample, provided by Genentech, using the same gradient with FA varied from 0.1% to 0.5%. A small amount of DFA or TFA with concentration of 0.01%, 0.025% and 0.05%, is further added to test its effect on LC resolution and MS sensitivity. RPLC temperature is also optimized to maximize selectivity and protein recovery. It is concluded that maximum LC resolution and maximum MS sensitivity of IgG1 are can be attained for this new bonded phase either with 0.25% FA + 0.025% DFA or with 0.5% formic acid. It is also found that lower temperature can be used to improve selectivity because protein recovery is improved by the polymer.

Keywords: HPLC, HPLC Columns, Liquid Chromatography/Mass Spectroscopy, Protein
Application Code: Bioanalytical
Methodology Code: Liquid Chromatography/Mass Spectrometry
Leukocyte enumeration is one of the most common clinical laboratory processes used for diagnosis and prognosis of a number of diseases such as leukemia, tuberculosis, any blood transfusion related complications such as transmission of cytomegalovirus. Conventional methods for cell isolation include filter based separation, density based centrifugation, biochemical markers or magnetic beads – FACS or MACS. However, these methods have drawbacks, such as physical clogging of the filters or unwanted activation of cell surface proteins due to use of biomarkers, several post separation washing steps, which make the recovery of the cells challenging. For these reasons, a size based approach for separation is desirable. Inertial microfluidics, which exploits the effects of both inertial and fluid viscosity in the micro-scale, for particle migration and sorting, has offered promising solutions to develop passive cell separation systems. In this work, we report a continuous, sheath flow aided, label-free technique for separation of leukocytes from erythrocytes, utilizing the inertial forces intrinsic to the microfluidic channel. Using sheath flow we are able to pre-focus particles in a virtually smaller microchannel prior to inertial separation downstream. We demonstrate the working parameters of the device using polystyrene beads prior to using blood. This inertial microfluidic chip is capable of separating Red Blood Cells from White Blood Cells from a diluted blood sample with a purity of 80% and recovery rate of WBCs of >90% at a sample flow rate of 30 µL/min and can be easily multiplexed to increase throughput as desired. This chip is also able to remove the excess sheath flow allowing to obtain a more concentrated sample at the WBC output. The high separation efficiency along with a versatile output design shows that this design can be integrated to other systems thus presenting the potential to develop a complete lab-on-chip platform.

Keywords: Biomedical, Biotechnology, Lab-on-a-Chip/Microfluidics
Application Code: Biomedical
Methodology Code: Microfluidics/Lab-on-a-Chip
Recent studies suggest that multiple forms of cancer can be detected by simultaneously studying kinase and protease expression patterns. [1] Improved outcomes can be achieved when cancer is detected in early stages, however at those stages the biomarker concentrations are low. Isoelectric focusing (IEF) has the ability to concentrate analytes from a large volume into smaller stable bands for detection. The separation is typically performed via a capillary electrophoresis instrument with detection systems that include chemical or pressure mobilization. This mobilization can perturb the separation and cause band broadening. To minimize the size, cost, and complexity of the system a microfluidic device was used with a scanning LIF with a PMT detector. A 3cm separation channel was scanned using a stepper motor attached to a lead screw that moved a 3D printed microfluidic chip carriage over the LIF detection point for the PMT. The short length of the channel allowed multiple scans of the device to be made while the electric field was applied. This allowed for greater optimization of the separation. A PDMS based chip with a separation channel of 3cm x300\mu m x16\mu m was used to separate and focus a series of fluorescently labeled peptides to be used as pI markers. The loading sample composition was optimized to reduce or eliminate EOF and ITP based drift of the focused bands. Also, multiple surfactants were studied to reduce the staining from the focused bands to allow the devices to be reused up to 8 times. Finally, protease activity was examined using peptide substrates whose fragments pI’s were tuned by adding charged amino acids during their synthesis to focus at specific pI’s.


Keywords: Bioanalytical, Enzyme Assays, Fluorescence, Lab-on-a-Chip/Microfluidics

Application Code: Biomedical

Methodology Code: Microfluidics/Lab-on-a-Chip
A novel ion sensing platform based on droplet microfluidics has been developed. A water-immiscible solvent dissolving the lipophilic ion sensing ingredients including chromoionophore, ionophore, and ion-exchanger is employed as an ion-selective oil phase. By using a T-junction or flow-focusing configuration, the oil phase merges with an aqueous sample phase and segments of organic and aqueous solvents are formed. Target analyte ions (e.g., Na\(^+\), K\(^+\), Cl\(^-\), and polyionic species in this work) in the aqueous segments are efficiently extracted into the oil segments because of their high binding affinity with ionophore molecules. To maintain electroneutrality of the oil phase, protons will be expelled from the oil (for cations) or extracted into the oil (for anions), which changes color and fluorescence of the chromoionophore that is detectable via a fluorescence microscope. Real physiological samples (e.g., serum) can be mixed with buffer on a chip and then merged with the oil phase. Compared to previous ionophore-based sensing configurations, the present platform has the following advantages: 1) response time is < 1s because of the enhanced diffusion of sensing molecules in polymer-free oil optode droplets and the favored interfacial transport of ions; 2) color or fluorescent detection from a vertical direction doesn’t suffer from optical interference from the sample matrix; 3) every oil segment is a fresh identical optode and thus sensor calibration is not necessary; and 4) screening of ion channel drugs could be achieved in an unprecedented throughput by introducing single cells into the aqueous segment.

**Keywords:** Biomedical, Clinical Chemistry, High Throughput Chemical Analysis, Lab-on-a-Chip/Microfluidics

**Application Code:** Biomedical

**Methodology Code:** Microfluidics/Lab-on-a-Chip
Although transplantation is the treatment of choice for patients with end-stage kidney failure, there is a tremendous organ shortage and a continuous high rate of discard. The main reason these organs are under utilised is because of the lack of tools to reliably assess their viability. Hence, marginal donor organs can be discarded although their function might not be impaired. Thus, developing tools to assess organ viability is a priority to safely extend the donor pool and increase the number of successful transplants. As the kidney needs to travel from the donor to the recipient site, continuous monitoring during transit would ensure that it could be safely transplanted upon arrival.

We are developing a fully portable wireless microfluidic biosensor system that can travel with the kidneys. On-line microdialysis is used to sample kidney extracellular fluid and the resulting dialysate is monitored for changes in levels of glucose and lactate, key metabolic markers of ischemia. We have built a new optimised high-resolution 3D-printed microfluidic flow-cell that securely mounts microelectrode-based amperometric biosensors [1]. The sensors are connected to a small battery-powered wireless potentiostat that transmits the currents in real time to an Android tablet via Bluetooth. The whole device is enclosed in a 3D-printed robust waterproof casing, allowing it to sit safely with the organ during transport. A custom-made Android application allows live display of the two signals on the tablet.

The challenges of developing the system will be presented, including monitoring over the wide temperature range encountered, together with preliminary results from porcine kidneys during hypothermic and normothermic machine perfusion. Results will be compared to those obtained using our older non-portable systems [2].

Separation of cells of interest directly from unprocessed whole blood is highly preferred as it not only offers extreme throughput but also minimizes cell loss and contamination from sample pretreatment. Although microfluidic devices have been widely explored for cell separation, preconditioning, such as dilution, is mandatory for hydrodynamic separation. Herein, we report on a novel approach to achieve cell focusing and separation directly from unprocessed whole blood based solely on cell size. The separation is achieved through careful engineering of sample-fluid configuration within a microchannel, which induces strong effect of shear-induced diffusion. Our results from high-speed imaging reveal that such effect coupled with fluid inertia rapidly focuses larger cells to the channel center for easy separation. Whole blood spiked with both fluorescent particles and Hep G2 cells were used to demonstrate the separation principle and its performance. No sample pretreatment and complex external controls were used in our device. The processing throughput can be as high as $10^7$ cells/sec in a single channel. Our results confirm the high performance in terms of efficiency (>90%) and RBC rejection rate (> 96%). In summary, we have successfully demonstrated the novel size-based cell focusing paradigm for direct separation of cells of interest from undiluted whole blood. We envision broad impact of our work on a wide range of applications, such as fast extraction of circulating tumor cells (CTCs) or blood fractionation.

Keywords: Biomedical, Biotechnology, Isolation/Purification, Lab-on-a-Chip/Microfluidics
Application Code: Biomedical
Methodology Code: Microfluidics/Lab-on-a-Chip
Epigenetic mechanisms leverage DNA three-dimensional structure to regulate gene expression. As a key example, post-translational modifications (PTMs) to histone proteins remodel DNA-histone associations and modulate transcription factors. Importantly, histone PTM dysregulation often correlates with disease such as lymphomas and solid tumors, but knowledge of these pathways has recently led to the first FDA-approved epigenetic therapies.

We are adapting Chromatin Immunoprecipitation (ChIP), a technique for linking histone PTMs to gene expression, into microfluidic droplets. By compartmentalizing samples into droplets surrounded by fluorinated oil, we will precisely handle picoliter sample volumes and reduce loss from contact with pipet or tubing walls that limit manual approaches to millions of cells. ChIP will become feasible for sample-limited systems such as small patient biopsies and rare cell populations.

In this work, we are developing a ChIP-in-Droplets system for isolating histone PTM species and associated DNA in droplets. We have engineered novel microfluidic approaches to control biochemistry inside each 200 pL droplet at high frequencies (200-600 droplets per second). For instance, we have characterized immunocapture of GFP-histone conjugates from modified HeLa by antibody-functionalized magnetic beads in droplets. Then, passing magnetic bead-containing droplets through our washing module uses an integrated magnet to purify targeted PTMs. For example, one pass through this module triples each droplet from 120 pL to 360 pL with water (dilution) then re-concentrates beads back to 120 pL during droplet splitting (selective capture). By performing these steps in series, washing operations dilute and remove material not bound to beads, collecting only the target PTM and DNA for downstream analysis. Ongoing work continues to optimize and integrate these systems for fully realizing ChIP-in-Droplets.

Support: NIH 1R21CA191186, NSF Graduate Research Fellowship

Keywords: Genomics, High Throughput Chemical Analysis, Immunoassay, Lab-on-a-Chip/Microfluidics
Application Code: High-Throughput Chemical Analysis
Methodology Code: Microfluidics/Lab-on-a-Chip
Microfluidics/Lab on a Chip - Biomedical

**Quantitative Measurements of Insulin Release From Single Islets of Langerhans in Response to an Anti-Diabetic Lipid**

The defective release of insulin from pancreatic islets of Langerhans disrupts glucose homeostasis in the body. It is therefore crucial to measure insulin quantitatively and with high temporal resolution. Although several systems have been described for high time resolution measurements, many are limited in their ability to quantify release. In this study, we describe a microfluidic analytical system that can both quantitatively monitor insulin secretion and maintain high time resolution and apply it to determine the effect of a novel anti-diabetic lipid.

Previous microfluidic systems for single islet hormone secretion measurements used pressure-driven perfusion systems to deliver glucose solutions to an islet chamber and sampled secretions by electroosmotic flow (EOF). Because of a discrepancy in these flow rates, only a small fraction of the secretions was sampled. Experimental variables, such as islet proximity to the sampling channel, can alter that percentage, hindering islet-to-islet comparisons of insulin measurements. Using finite element analysis, a microfluidic system was designed that ensured cellular secretions were homogenized (RSDs < 3%) prior to sampling, permitting quantitative monitoring of insulin and examination of inter-islet biological variability. Using the new design, the system was tested with standard insulin solutions and demonstrated a detection limit of 10 nM insulin. The system was then used to determine the effects of a newly-discovered anti-diabetic lipid, fatty acid hydroxy fatty acid. The lipid was acutely and chronically administered to islets, and was found to augment insulin release under both conditions, unlike free fatty acids which impaired insulin secretion after chronic exposure.

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

**Application Code:** Bioanalytical

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

Development of a Label Free Microfluidic Platform for Directed Enzyme Evolution

The reactivity and selectivity of natural enzymes often surpass the capabilities of synthetic methods for the production of complex molecules. Adaptation of enzymes to industry has allowed cheaper and more efficient production of chemicals ranging from pharmaceuticals to biofuels. Adapting these biocatalysts to perform on non-native substrates presents a challenge to researchers, who must screen through thousands of modified enzymes to identify mutations favorable to biorthogonal catalysis. Droplet microfluidics provides an attractive approach to this form of directed evolution. Established techniques for the generation and sorting of compartmentalized samples allows the expression of millions of enzyme variants within individual picoliter to nanoliter scale droplets. To date, these large libraries have only been screened using optical readouts, which limits the range of enzyme products that can be detected. We are developing a system that overcomes this limitation by using mass spectrometry to analyze the contents of these droplets. In this system, individual magnetic beads are coated with copies of a unique DNA sequence that encode an enzyme variant. These beads are isolated in 2 nL droplets containing the necessary components for in-vitro expression. Empty droplets are removed via magnetic sorting, and droplets are reinjected and dosed with substrate after incubation. These droplets are then split and the magnet free portion is analyzed with nanoelectrospray ionization-mass spectrometry, whose nanomolar limits of detection will allow for label-free analysis of the enzymatic variants, even at low levels of turnover. Analysis of droplets as small as 300pL has been achieved at up to 6 Hz, suggesting the potential to screen tens of thousands of enzyme variants and condense months of resource intensive experimentation into a single test tube and a single day.

This work is supported by NSF GOALI award 1604087 and NIH training grant NIBIB T32 EB005582.

Keywords: Electrospray, High Throughput Chemical Analysis, Lab-on-a-Chip/Microfluidics, Mass Spectrometry
Application Code: High-Throughput Chemical Analysis
Methodology Code: Microfluidics/Lab-on-a-Chip
Molecular Spectroscopy

Single-Shot Microsecond-Resolved Spectroscopy of the Bacteriorhodopsin Photocycle with Quantum Cascade Laser Frequency Combs

We present the IRspectrometer, a quantum cascade laser dual frequency comb spectrometer [2-3]. It allows for parallel acquisition of hundreds of mid-infrared wavelengths with microsecond time resolution. The formation of the light-activated L, M and N-states in bacteriorhodopsin – which only have µs to ms lifetimes – has been recorded with the setup shown in Figure 1 a). Figure 1 b) illustrates the infrared response of bacteriorhodopsin to 10 ns visible light pulses with microsecond time-resolution. The different wavelengths were all measured in parallel thanks to the dual-comb approach. The spectra as well as the kinetics show good agreement with those from step-scan FT-IR measurements. As a benchmark, the spectral signature of several intermediate states of the bacteriorhodopsin photocycle has been recorded in a single shot measurement. This approach greatly reduces the complexity of time-resolved bio-spectroscopy measurements in the mid-infrared which currently require many repetitions.

REFERENCES

Abstract Text
Time-resolved vibrational spectroscopy is an important tool for understanding biological processes and chemical reaction pathways [1]. Today, all available methods to our knowledge require many repetitions of an experiment to acquire a microsecond time-res. mid-IR spectrum.

Keywords: Molecular Spectroscopy, Protein, Spectroscopy, Vibrational Spectroscopy

Application Code: Bioanalytical

Methodology Code: Molecular Spectroscopy
Microfluidic paper-based analytical devices (PADs) are very helpful for chemical and biochemical detection and can enable point of care testing allows for patient diagnoses in hospitals, in field, in ambulances, in the physician’s office an even at home. While most PADs make use of colorimetric or electrochemical methods to produce a change of color or generate a current and/or potential in response to the presence of an analyte, we are developing a PAD with chemical separation and Surface enhanced raman scattering (SERS) capabilities. SERS enhancement of molecules on the PAD enables chemical identification of the analytes in a complex sample. The combination of SERS capabilities with PADs provides higher levels of signal amplification for detection and quantification of several analytes. SERS is obtained from a high surface area Ag nanostructured dendrites made by electrodeposition into the paper. Modification of the length and shape of the microfluidic channel printed onto the paper affect the sensitivity of the device. Our device was optimized to work with samples volumes as small as 2 μl. The combination of SERS-PADs and portable Raman spectrometer will provide inexpensive chemically selective detection of analytes in mixtures.
Surface enhanced Raman spectroscopy (SERS), provides non-destructive, label-free, chemical-specific identification of molecules that interact with SERS-active nanostructures. SERS is able to address challenges associated with more commonly used NMR and MS methods. Thus, SERS provides complementary molecular identification and more complete coverage of molecules detectable in complex samples. Here we demonstrate a high-throughput coupling of liquid chromatography with SERS (LC-SERS) using an online sheath-flow SERS detector for analysis of isomeric phosphorylated sugars such as glucose 1-phosphate, glucose 6-phosphate and fructose 6-phosphate. This sheath-flow SERS interface utilizes hydrodynamic focusing to confine the analytes eluting out of the column onto a planar hexanethiol SAM on silver SERS-active substrate where the molecular intrinsic SERS signals are detected. This methodology is capable of detecting and quantifying phosphorylated sugar isomers over a wide range of concentrations and in complex biological matrices. Our work suggests a new route to identifying these isomeric molecules for diagnostic assays which still remains challenging for other detection methods.
Transformer insulation oil when subjected to abnormal conditions degrade, resulting in the formation of gases like hydrogen, methane, acetylene, ethylene, ethane, carbon monoxide and carbon dioxide. These gases are trapped in a chamber in the Buchholz relay. The free gas analysis can diagnose electrical faults or types of isolation that have occurred and allow for targeted interventions, avoiding high costs of maintenance, as well as more serious problems in the transmission of energy. A simple analytical procedure using near-infrared spectroscopy with Fourier transform and multivariate techniques for the rapid determination of free gas in power transformers was developed. A set of 85 samples of gas mixtures were prepared from a standard mixture. A volume of 200 ml of each sample was inserted with the aid of a gastight syringe on gas cell with optical path of 2.5 m and was spectral scanning between 7500-5000 cm\(^{-1}\) (Figure 1). The predictive models were developed using the second derivative (Figure 2) and partial least squares regression (PLS). For the set of 70 samples were used and calibration 15 samples for the validation set. The results of the models presented very good correlations with the highest correlation coefficient 0.9977 and lower 0.9678, and root-mean-square of 4.62% prediction and 5.84% respectively. The 15 samples analyzed by the model showed values very close to chromatographic results. The technique proved efficient on the free gas analysis so fast, accurate, non-destructive and simultaneously, reducing time and costs, making possible the analysis of a larger number of power transformers to ensure a more effective control of the life of this equipment.

Keywords: Gas, Gas Chromatography, Method Development, Near Infrared
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Molecular Spectroscopy
Nanoparticle analysis at single particle level can reveal the particle-to-particle heterogeneity beyond the bulk or average properties achieved from conventional nanoparticle measurements. The use of a high bandwidth and low noise electrochemical method indicates a more direct way to explain the fast heterogeneous electron-transfer behavior of redox reaction at high fidelity. Early research of this topic was mainly focused on using planar ultramicroelectrode due to its easy fabrication process. In recent years, smaller electrodes with more complex nanostructure, e.g. nanopore or nanotip, were prepared to provide confined space and additional interaction between the particle and electrode. Herein, we fabricated nanopore electrode arrays with two closely spaced and individually addressable electrodes in the vertical direction. The top ring electrode served as the particle gate to control the nanoparticle transport within nanopore arrays. And then the collision events of nanoparticles were collected by the bottom disk electrode. For the first time, we observed the voltage-gated nanoparticle transport inside nanopore electrode arrays, mimicking the ion transport through the ion channels embedded in the cell membrane. In detail, there is a voltage threshold for nanoparticle accessing to nanopore electrode, i.e. a higher enough potential was required to observe largely increased collisions on the bottom electrode. And the voltage threshold was strongly dependent on the applying potential of both electrodes, the size of nanoparticle and the surface charge of nanopore. Overall, we believe this study provides a delicate way to in-situ monitor the nanoparticle transport and reactions within nanoconfined space at single particle level.

Keywords: Electrochemistry, Microelectrode, Nanotechnology, Particle Size and Distribution
Application Code: Nanotechnology
Methodology Code: Electrochemistry
It is well known that membranes with conically shaped pores (Figure 1) can rectify ion current flowing through the pores from electrolyte solutions on either side of the membrane. In order for the ion current to be rectified in this way, the pore walls must be charged, for example by carboxylate groups on the pore walls, and the tip opening of the pore must be small (~10 nm). We recently showed that because ion-current is rectified, electro-osmotic flow (EOF) through the membrane is likewise rectified. This allowed us to develop a new type of EOF pump that yields net flow through the membrane, in the direction from base opening to tip opening (Figure 1), when a symmetrical sinusoidal voltage wave is applied across the membrane.

In this talk we build on this AC EOF pump concept. Since the pumping action requires surface charge, we reasoned that if adsorption of an ion could modify the extent of that surface charge, then the pump could either be turned on or turned off in the presence of that ion. To prove this concept we used simple EDC coupling chemistry to attach aminomethyl-18-crown-6 to the carboxylate groups on the pore walls of a conical pore poly(ethylene terephthalate) membrane. The extent of coupling was controlled such that not all the carboxylates were utilized yielding membranes that still had anionic surface charge.

18-crown-6 strongly binds Pb2+, so exposure of these membranes to dilute aqueous Pb2+ causes the introduction of positive surface charge. This effectively lowers the amount of excess negative charge, and the EOF flow rate was observed to decrease upon exposure to Pb2+. We have shown that over the Pb2+ concentration range from 100 nM to 100 μM the flow rate decreased from 2.75 L/min to zero. Hence, this is a smart pump that turns off when it encounters sufficiently high (100 μM) concentrations of lead.
Graphene and metal oxide based nanomaterials offer remarkable results in energy storage and harvesting applications. High surface area and porous material serve as excellent electrode in energy storage applications. Thus, CVD grown multiwall carbon nanotube on reduced graphene oxide (rGO) coated Ni-foam was used in supercapacitor. This nanostructured PANI/MWCNT grown on rGO coated nickel foam offered high surface area and good capacitance value of 587 F-g⁻¹. Efficient cyclic stability was achieved within 1 hr at a scan rate of 20 mV-s⁻¹. Synthesis of porous zirconia-reduced graphene oxide (ZrO₂-rGO) nanocomposite was also carried out and study of its hydrogen storage behaviour was done at room temperature. ZrO₂-rGO nanocomposite illustrated a large surface area of 390 m²g⁻¹ and mesoporous structure with average pore width of 2.56 nm. The hydrogen sorption behaviour of this nanocomposite was confirmed by electrochemical studies. Thin film was formed of reduced graphene oxide nanoribbons (rGONRs) embedded in polyvinyl difluoride (PVDF) polymer matrix which was used as triboelectric nanogenerator. rGONRs/ PVDF single electrode triboelectric nanogenerator (TENG) gave output by contact separation mode. The output voltage of TENG was found to be 0.35 V which was stored and could be used for lighting up the LED. Anatase TiO₂ plants were synthesized by hydrothermal method which resulted into a water chestnut like morphology. The TiO₂ plants hierarchical nanostructure exhibit excellent photocatalytic performance when applied in photo degradation of Rhodamine B organic dye. These unique features make them a promising candidate in field such as solar cells, photocatalysis, supercapacitor, lithium ion batteries and some related fields. Hence the synthesis of these new nanomaterials prove very beneficial in the field of energy storage and harvesting.
Abstract Title: Engineering of Functional Oxide Nanostructures for Solar Energy Conversion

Primary Author: Gobinda G. Khan  
Tripura University (A Central University)

Abstract Text:
The photoelectrochemical (PEC) cells, which exhibit significant promise in the direct conversion of enormous solar energy into electricity and chemical fuels (hydrogen and hydrocarbons) is a remarkable approach to reduce environmental pollution caused by fossil fuels and the proper utilization of renewable and green energy source. Here, highly oriented arrays of surface functionalized/surface doped ZnO Nanorods and CuFeO2/ZnO nano-heterostructures, fabricated by a simple wet chemical method, were employed as the efficient photoanodes for water splitting under the visible solar light. Surface functionalization significantly improved both the photocurrent and photostability of the ZnO Nanorods and the CuFeO2/ZnO nano-heterostructures electrodes under visible-light irradiation along with the reduction in onset potential for water oxidation. Surface engineered ZnO Nanorods also exhibited enhanced visible light harvesting efficiency and significantly quenched electron-hole recombination leading to highly enhanced carrier separation. Surface engineering remarkably tuned the electronic structure of the photoanodes changing the band position and band bending at electrode-electrolyte interface leading to low electrical resistance and fast charge transportation of the device boosting the PEC property. The study demonstrates that the surface functionalization is an easy and general approach for functional oxide semiconductors to achieve an effective solution for high performance solar light-driven water splitting.

Keywords: Electrochemistry, Electrode Surfaces, Fuels\Energy\Petrochemical, Nanotechnology

Application Code: Nanotechnology

Methodology Code: Electrochemistry
Sialic acid-binding immunoglobulin-type lectins, or Siglecs for short, are a class of transmembrane glycoproteins that are involved in cellular recognition primarily in the immune system, but also notably in the nervous system. In humans there are dozens of known Siglecs, and each of them binds specific sialic acid linkages found on glycoproteins or glycolipids, such as gangliosides. Siglec-4, also called myelin associated glycoprotein (MAG) is the only known Siglec found in the nervous system. MAG is a potent inhibitor of neuron growth, and soluble MAG associated with neurodegenerative disorders like multiple sclerosis inhibits nervous system repair. Gangliosides on the axon are known to be ligands for MAG, however systematic studies of the binding kinetics and affinities between MAG and gangliosides are lacking quantitative characterization. In this work we immobilize MAG on surfaces, either glass or supported bilayers, and monitor the binding of ganglioside-containing vesicles using quartz crystal microbalance and TIRF microscopy. By counting individual binding MAG-vesicle binding events and monitoring their bound state lifetimes we are able to determine binding kinetics and affinities for MAG-ganglioside interactions. We find that gangliosides GT1b and GD1a interact with MAG significantly more strongly than does GM1, and that membranes lacking gangliosides do not interact significantly with MAG. Additionally we are investigating the modulatory effect of cholesterol on MAG-ganglioside interactions.
Hydrogen peroxide (H\(_2\)O\(_2\)) is a reactive oxygen species that is implicated in many neurodegenerative diseases. It participates in normal modulation of cell function; however, it can also contribute to oxidative stress, neuronal dysfunction, and cell death. H\(_2\)O\(_2\) is membrane permeable, allowing for it to diffuse through cells and into the extracellular space following its formation. There is a critical gap in understanding the unique role H\(_2\)O\(_2\) plays within the brain, as dynamic changes in concentrations of extracellular H\(_2\)O\(_2\) in living tissue are not well known. Previous attempts to understand this have been hindered by insufficient detection sensitivity and selectivity with conventional analytical techniques. However, fast-scan cyclic voltammetry (FSCV) allows for reliable detection of rapid fluctuations in H\(_2\)O\(_2\) on a sub-second timescale. This experiment utilizes FSCV coupled with carbon-fiber microelectrodes in intact brain tissue to investigate striatal H\(_2\)O\(_2\) fluctuations with changes in dopaminergic pathway activity, elicited by electrical stimulation. A novel double waveform has been developed to facilitate removal of interference from sources that generate background subtracted current at potentials near the peak oxidation potential of H\(_2\)O\(_2\), such as shifts in pH. Striatal H\(_2\)O\(_2\) oxidation was monitored following electrical stimulation of dopaminergic fibers that innervate the recording region. Stimulations were delivered at varying frequencies (15, 30, 60, 90, and 120 Hz) using increasing pulse numbers. The data demonstrate that changes in the H\(_2\)O\(_2\) signal correspond with the duration of the stimulation. The results will elucidate the dynamic relationship between H\(_2\)O\(_2\) and dopamine in the striatum in response to neuronal activation.
Thanks to advances in chemotherapy and other treatments, more and more people are surviving after being diagnosed with cancer. Chemotherapy-induced cognitive impairment ('chemobrain') is a syndrome that results in the impairment of executive function. This work employs fast-scan cyclic voltammetry (FSCV) at carbon-fiber microelectrodes to investigate sub-second changes in dopamine levels while rats are carrying out behavioral paradigms designed to assess cognitive impairment. The behavioral paradigms are tailored to measure deficiencies in specific components of executive function. Here, we focus primarily on the task of inhibition, assessed by the differential reinforcement of low rates paradigm. Our results show that dopamine levels, measured in the dorsal striatum, are maintained at a high level during increased cognitive load, but decrease after the rat presses the lever. Comparisons between chemotherapy-treated rats and saline treated rats will be discussed.

Keywords: Bioanalytical, Electrochemistry, Neurochemistry
Application Code: Neurochemistry
Methodology Code: Electrochemistry
Parkinson's disease (PD) is a neurodegenerative disease characterized by the slow degeneration of dopaminergic neurons found in a region of the midbrain called the substantia nigra. Dopamine (DA) plays a key role in regulating motor function. Thus, the destruction of these neurons and the consequential decrease in DA concentrations in the striatum leads to the deterioration of motor control. The drug Levodopa has been used to treat PD by helping to increase the concentration of DA in the brain. This drug has been proven to alleviate the motor symptoms of PD; however, after a short period of time, dyskinetic symptoms can develop. Treatment with Citalopram, a serotonin reuptake inhibitor, before administration of Levodopa has proven to attenuate the dyskinesia side-effects. Furthermore, it is thought that oxidative stress is a principal contributor to the destruction of dopaminergic neurons, and possibly to the development of dyskinesias, in PD and its treatment. To date, oxidative stress has been difficult to measure due to the high reactivity of oxygen radicals; however, hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}) can serve as an indicator of the presence of oxidative stress. This experiment uses fast-scan cyclic voltammetry coupled with carbon-fiber microelectrodes to simultaneously monitor real-time fluctuations of DA and H\textsubscript{2}O\textsubscript{2} in the dorsal striatum. These neurochemical dynamics can be time-locked to dyskinetic episodes. Overall, these studies will aid in our understanding of how oxidative stress modulates nigrostriatal DA signaling, as well as the behavioral consequences of this interaction. The results will inform improved therapeutic strategies for the treatment of PD.

**Keywords:** Electrochemistry, Microelectrode, Neurochemistry, Voltammetry

**Application Code:** Neurochemistry

**Methodology Code:** Electrochemistry
Neurochemistry - Normal and Pathological Brain Measurements

Detection of Glucose and Potassium in Cortical Spreading Depolarizations After Traumatic Brain Injury in Rats Using Continuous Online Dexamethasone Enhanced Microdialysis

Traumatic brain injury (TBI) represents a substantial health crisis throughout the world. Monitoring secondary injury in the days following the initial damage, including spreading depolarization (SD), is a significant analytical challenge. SDs are waves of neuronal depolarization, followed by an increased energy demand to repolarize. Monitoring glucose and potassium as markers of SD waves can be accomplished by microdialysis. However, immune response to the penetration injury caused by probe implantation greatly diminishes the temporal sampling window. Previous work from our lab has established that five days of retrodialysis with the dexamethasone mitigates immune response to the probe itself and increases the functional lifetime of the probe.

Controlled cortical impact (CCI) rats, as a model for TBI, and continuous online microdialysis were employed to monitor glucose and potassium levels. A continuous online microdialysis system developed by the Boutelle group coupling a potassium ion selective electrode with 30 second temporal resolution glucose sampling was employed to monitor potassium and glucose simultaneously. As a result, glucose and potassium levels were monitored continuously out to ten days after probe implantation. SD was measured in both CCI animals and sham controls, and histology was used to confirm a healthy interface between the probe and the surrounding tissue.

Keywords: Bioanalytical, Biosensors, Electrochemistry, Neurochemistry
Application Code: Neurochemistry
Methodology Code: Sensors
Cerebral blood flow (CBF) controls the delivery of glucose and oxygen to the brain and the removal of waste. Blood flow is increased when brain tissue is activated in a phenomenon known as functional hyperemia. Currently, either optical techniques or fMRI is utilized to monitor CBF. This presentation will discuss efforts to use microfabricated sensor employing electrolytic hydrogen clearance (EHC) technique to monitor changes in blood flow in the brain. In EHC, hydrogen is generated galvanostatically by an electrode and its clearance monitored by a separate electrode. The rate of clearance can be correlated with physical processes that alter its local concentration, such as diffusion and blood flow. In this work, a microfabricated sensor comprised of a pair of platinum electrodes is utilized for making EHC measurements. Microfabricating the sensor enables small probes to be fabricated, small enough to enable use deep in the brain. This sensor has been characterized ex vivo, demonstrating its operation. More recent efforts have transitioned the sensor to tissue to monitor blood flow in the cortex and in the striatum during pharmacology.

Keywords: Bioanalytical, Neurochemistry
Application Code: Neurochemistry
Methodology Code: Electrochemistry
Transient Adenosine Changes During the Early Stages of Ischemia and Reperfusion Injury

Adenosine is an important neuromodulator in the central nervous system, which plays a vital role in variety of physiological and pathophysiological processes. Previous studies have shown that tissue adenosine levels increase dramatically during the ischemic injury and attenuate the excitotoxic neuronal injury. Our lab has developed an electrochemical fast-scan cyclic voltammetry (FSCV) method recently, that identifies rapid, spontaneous changes in adenosine concentrations that last only about 3 seconds. In this present study, we focused on understanding how the transient adenosine release frequency and concentration changes during the early stages of ischemia and reperfusion injury. Transient adenosine measurements were carried out in the caudate-putamen of the anesthetized rats continuously, for a two hour period of normoxia, followed by 30 min of ischemia through bilateral common carotid artery occlusion and 90 min of reperfusion. This study suggests that, transient adenosine frequency was increased after the induction of ischemia and remained elevated during reperfusion. The total number of adenosine transients increased by 52% during ischemia and reperfusion periods combined, compared to normoxia. The cumulative adenosine concentration was increased by 53% during ischemia and reperfusion periods compared to normoxia. Further, we assessed the role of A2A antagonist, SCH442416, a putative neuroprotective agent to affect adenosine transients. SCH442416 significantly decreased the transient frequency during ischemia-reperfusion by 27% and the cumulative concentration by 31%. Our results demonstrate that this mode of rapid adenosine release increases during early stages of ischemia-reperfusion injury and it could provide fast, local neuromodulation and neuroprotection during ischemia.

Keywords: Bioanalytical, Electrochemistry, Microelectrode, Neurochemistry

Abstract Text

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Traditionally, it has been thought that glucose is the principal energy source of the brain. Recently, this widely accepted concept has been challenged by several studies demonstrating lactate as an important molecule with an essential role in energy metabolism and memory formation. As such, real-time molecular detection of lactate dynamics is imperative to understanding brain energy availability, and its involvement in neuropathological disorders such as Alzheimer’s disease.

However, to date, existing methods for detecting brain lactate concentrations are limited in terms of temporal and spatial resolution. We have addressed this need by developing and characterizing a novel lactate oxidase-modified carbon-fiber microbiosensor and coupling it with fast-scan cyclic voltammetry. This approach enables detection of rapid lactate fluctuations with unprecedented spatiotemporal resolution as well as excellent stability, selectivity, and sensitivity at discrete recording sites in the rat striatum. It can be coupled with our previously developed glucose-oxidase microbiosensor to enable simultaneous detection of both essential non-electroactive molecules. Combined, these new tools enable quantitative investigation of limitations to brain metabolism in disease states.

Keywords: Biosensors, Biotechnology, Electrochemistry, Voltammetry
Application Code: Bioanalytical
Methodology Code: Sensors
Selective serotonin reuptake inhibitors (SSRIs), prescribed for depression, are thought to act by blocking serotonin reuptake via inhibition of serotonin transporters (SERTs), though many questions remain on the precise mechanism of action, dose regimen, and time course surrounding this treatment. We aim to elucidate the mechanisms behind the SSRI efficacy using two methods: 1) [i] in vivo [/i] fast-scan cyclic voltammetry (FSCV) of serotonin in the mouse hippocampus to determine the voltammetric dose response of escitalopram, a popular SSRI, and 2) [i] in vitro [/i] confocal laser-scanning microscopy of a fluorescent SERT substrate, ASP+, that mimics serotonin uptake in stem-cell derived serotonergic neurons to determine the function of SERTs after escitalopram administration in both active and resting neurons. Our [i] in vivo [/i] results indicate that after acute, systemic SSRI administration, evoked serotonin release and reuptake in the hippocampus changes dynamically in a dose-dependent, but not linear, manner. Interestingly, for intermediate doses, serotonin reuptake 5 minutes post SSRI is [i] increased [/i] relative to control. To understand this unexpected increase in the rate of serotonin reuptake, we studied SERT function [i] in vitro [/i] in both resting and active serotonergic neuronal stem cells. After acute exposure to SSRI, we observed a difference in SERT function between active and resting neurons, with active neurons exhibiting increased functionality at 5 minutes, relative to resting neurons, possibly attributable to increased surface expression. Taken together this [i] in vivo [/i] and [i] in vitro [/i] data show that the function and expression of SERT after acute SSRI administration is complex and dynamic. In the future, this information can be employed to improve understanding of SSRI efficacy, aiding the development of better therapeutics for depression.

Keywords: Bioanalytical, Electrochemistry, Fluorescence, Microscopy
Application Code: Neurochemistry
Methodology Code: Electrochemistry
Neuroinflammation, both acute and chronic, has been predicted to cause dysregulation in the central nervous system (CNS) of healthy individuals. Of relevance to our work, neuroinflammation is predicted to play a role in or increase the risk for depression. The growing rate of depression highlights the importance of discerning the link between inflammation and this disorder. Specifically, we hypothesize that histamine, a key marker of inflammation, modulates serotonin, implicated in the monoamine hypothesis of depression. We have studied this by characterizing acute and chronic inflammation in mouse and rat models, which also display depressive-like behaviors. Acutely, inflammation is induced using a peripheral injection of lipopolysaccharide (LPS) which induces a rapid, potent systemic immune response. Chronically, inflammation is also observed in a high fat diet (45 kcal % fat) rodent model of obesity. In each model, we evaluate the histamine and its co-modulation of serotonin with employing fast-scan cyclic voltammetry and serotonin with fast-scan controlled adsorption voltammetry. Results suggest that inflammation increases histamine signaling and via its modulation of the serotonin system, causes selective serotonin reuptake inhibitors, common antidepressants, to be less effective.
The selective targeting of specific neuronal subtypes using genetic techniques such as chemogenetics has facilitated the understanding of the functional roles and mapping of complex brain circuits. Chemogenetics allows for the excitation or inhibition of neuron firing through viral transduction of Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) that are activated by a pharmacologically inert ligand, Clozapine-N-Oxide (CNO). However, it is not well studied how activation of excitatory or inhibitory DREADDs affect neurotransmitter dynamics (release and clearance). In this study, we used a viral targeting system to restrict DREADD expression to catecholamine neurons and employed in vivo fast-scan cyclic voltammetry (FSCV) to determine how CNO modulates dopamine (DA) and norepinephrine (NE) release in anesthetized rats. Furthermore, we utilized a retrograde intersectional viral approach to selectively modulate specific DA and NE pathways. Through immunohistochemical and electrochemical evidence, we demonstrate viral targeting of a subset of catecholamine neurons and determined that CNO dose-dependently activates DREADDs, leading to excitation and/or inhibition of neurotransmitter release. These results from incorporating in vivo FSCV with chemogenetics will provide the fundamental framework to understand how local catecholamine circuits are linked to various brain functions as well as psychiatric and neurodegenerative diseases.
Neurochemistry of Serotonin and Dopamine

The Dynamics of [i]In Vivo[/i] Serotonin Chemistry in Different Brain Regions

Serotonin is an important neuromodulator in the brain, implicated to underlie behavioral deficits of various psychiatric disorders. The treatment of mood disorders presents a challenge due to the poor characterization of serotonin chemistry in healthy and in disease models. In particular it is important to characterize serotonin chemistry in different brain regions, since separate localities are thought to be more involved in specific diseases. To address this shortcoming, in this paper, we characterized five novel voltammetric stimulation measurements pathways. Specifically, we measured evoked (fast scan cyclic voltammetry, FSCV) and basal (fast scan adsorption controlled, FSCAV) serotonin in the mouse prefrontal cortex, hippocampus, amygdala, habenula and striatum - regions with importance in a variety of disorders including depression, autism spectrum disorder and addiction. The dynamics of serotonin release and reuptake, in addition to ambient levels, exhibit distinctive differences between these regions. To gain further insight into the extracellular mechanisms that regulate serotonin in these areas, we mathematically modeled our responses. The models show that substantially different mechanism serve to control serotonin in these brain regions. Our study is the first to use this powerful combination of tools to obtain information on the unique regional differences exhibited by the serotonergic system.

Keywords: Bioanalytical, Electrochemistry, Microelectrode, Neurochemistry

Abstract Text

Bioanalytical, Electrochemistry, Microelectrode, Neurochemistry

Application Code: Neurochemistry

Methodology Code: Electrochemistry
Neurochemistry of Serotonin and Dopamine

Pain in Anesthetized Animals Causes Decreased Tonic Dopamine Levels Resulting in Increased Phasic Signaling

Pain is an enormous expense for our country costing $560-$635 billion annually. Therefore, it is important that we understand the neurochemical basis for pain to better mitigate its negative consequences. This understanding can be enhanced by techniques that measure neurotransmitter release on multiple temporal regimes. Specifically, tonic (steady-state, > minutes) and phasic (rapid firing, < 10 seconds) are two neurotransmission regimes of interest. Fast-scan cyclic voltammetry (FSCV) and fast-scan controlled-adsorption voltammetry (FSCAV) allow for quantification of phasic and tonic dopaminergic neurotransmission, respectively. Dopamine in the mesolimbic pathway is involved in encoding salient stimuli and it is hypothesized that tonic levels regulate phasic signaling. With FSCV and FSCAV, we determined how tonic neurotransmission regulates phasic signaling in response to pain in the nucleus accumbens shell (NAcs). We applied capsaicin, which binds the TRPV-1 pain receptor, to the cornea of anesthetized animals and measured changes in tonic and phasic dopamine. FSCAV measurements made every twenty seconds show a decrease in tonic dopamine in response to capsaicin. The observed decrease occurred independently of whether the application of capsaicin was ipsilateral or contralateral to the hemisphere of dopamine measurement. FSCV experiments indicate a corresponding increase in electrically evoked phasic dopamine release after the same painful stimulus. The increase in evoked phasic release lasts only for the duration of tonic decrease. The compatibility of FSCV and FSCAV, along with their impressive temporal resolution allowed us to show directly that acute pain induces a hypodopaminergic state in the NAcs, which results in increased phasic release. We conclude that tonic dopaminergic signaling regulates phasic firing in response to acute pain.

Keywords: Electrochemistry, Microelectrode, Neurochemistry
Application Code: Neurochemistry
Methodology Code: Electrochemistry
There is increasing public concern over the growing issue of pesticides. Many pesticides function by disrupting key neurochemical messengers in pests. These mechanisms raise the question of the impact of pesticides on the human brain. Despite the advancement and development of techniques to analyze neurotoxicity, the hazard pesticides bring to the human brain remains unclear. In this work, we apply fast-scan cyclic voltammetry to measurements of serotonin and histamine in mice that have been exposed to chlorpyrifos, a hallmark organophosphate pesticide. We explored the effects on neurochemistry of different doses, exposure routes and exposure times of this chemical. The general trend is that chlorpyrifos reduced serotonin transmission in exposed mice. Exposure to organophosphates, such as chlorpyrifos, has previously been thought to induce neuroinflammation. We hypothesize histamine levels in the brain to increase during neuroinflammation and since we recently observed that histamine negatively modulates serotonin, we test the notion that chlorpyrifos’ effects on serotonin are via histamine receptors. This study will allow a greater understanding of the neurotoxicological effects of pesticide exposure on the human brain.

Abstract Text
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Keywords: Electrochemistry, Neurochemistry, Pesticides, Voltammetry
Application Code: Neurochemistry
Methodology Code: Electrochemistry
It is known that weak base small molecule drugs can accumulate in acidic organelles such as synaptic vesicles by acidic trapping. Recently, it has been shown by optical methods that the antipsychotic drug cyamemazine accumulates in synaptic dopamine vesicles and is released upon electrical stimulation. In the present study, we employ fast scan cyclic voltammetry in acutely prepared rat brain slices to measure the effects of the vesicular release of cyamemazine on evoked dopamine responses. We have observed that under conditions designed to promote the vesicular release of cyamemazine (which consist of a long period of loading the brain slice with the drug, followed by bipolar optically isolated electrical stimulation), evoked dopamine responses can be increased in duration by an order of magnitude. To date, this effect has been detected in about 50% of the experiments. Interestingly, the first attempt to elicit the effect at a given recording site does not always work, but sometimes a subsequent stimulus can elicit the response. This observed response appears similar to the effect that is seen upon administration of drugs which are known to inhibit the dopamine transporter and thereby impede the reuptake of dopamine. However, unlike with uptake inhibitors, but consistent with an effect mediated by the release of a substantial vesicular “charge” of the drug, this effect is not present for subsequent stimuli. To our knowledge, this is the first evidence supporting the hypothesis that drug action can be mediated by its vesicular release. We anticipate that this finding will give new insights into both the mechanistic functioning of the dopamine system and the action of antipsychotic drugs. This research is supported by an NIH grant, R21 MH110153.

Keywords: Electrochemistry, Microelectrode, Neurochemistry, Voltammetry
Application Code: Neurochemistry
Methodology Code: Electrochemistry
Neurochemistry of Serotonin and Dopamine

Evaluating the Fundamental Serotonin Chemistry of the Prefrontal Cortex in the Context of Autism Spectrum Disorder

Autism spectrum disorder (ASD) is a collection of developmental disorders with growing prevalence. The pathophysiology of ASD is not yet fully understood, hindering suitable prevention and treatment options. Specifically, a universal underlying chemical mechanism is lacking. We believe that serotonin dysfunction can be identified as a common neurochemical mechanistic feature of this disorder, however current techniques do not provide a complete representation of the serotonin system as they are only capable of measuring basal levels at low temporal resolution. Here we describe the application of Fast-Scan Cyclic Voltammetry, which operates on a neurotransmission temporal resolution, to examine serotonin release and reuptake in the medial prefrontal cortex of genetically engineered mouse ASD models. These models allow us to establish a chemical phenotype accompanying stereotypical ASD behaviors in mice. The results demonstrate a significant difference in the serotonin chemistry between ASD models and controls. Identifying this chemical phenotype will allow us to redefine the serotonin chemistry within ASD.

Keywords: Electrochemistry, Lead, Neurochemistry, Voltammetry
Application Code: Neurochemistry
Methodology Code: Electrochemistry
The availability of on-site assay technologies that deliver early and accurate identification of pathogens is of critical importance. Recent disease outbreaks and increased food recalls due to bacterial contamination exemplify this urgent need. Our work directly addresses the current lack of simple, rapid, sensitive, and selective pathogen detection methods needed for frontline intervention in the most at-risk populations. We developed a novel tuberculosis (TB) biosensing system in both microtiter plate and paper-based platforms. The dual-platform design afforded either quantitative (microtiter plate, 1.0-20.0 nM) or qualitative (paper) detection. We extended the paper-based platform concept to Epstein-Barr virus (EBV) detection using a target-bridged capture scheme in which EBER-1 RNA from EBV linked a tethered probe to a fluorescent reporter probe for a low nanomolar detection limit. Additionally, we have incorporated isothermal DNA amplification technologies with paper-based detection of Zika virus. While divergent in design and target, these biosensing systems achieve the aims of current pathogen detection research while providing cost-effective options for deployment in any locations.

Keywords: Analysis, Bioanalytical, Biomedical, Nucleic Acids
Application Code: Biomedical
Methodology Code: Sensors
In hydrocephalus, cerebrospinal fluid (CSF) builds up in the cranial cavity, causing swelling of the head and potentially, brain damage. A shunt to drain the fluid into a body cavity is now universally used, but shunt failure is all too common. Techniques for ascertaining shunt failure are time-consuming, expertise-dependent, and often inconclusive. We report an inline system that reliably and quantitatively measures the CSF flow rate. The system uses a single thermistor to both heat the surrounding and to sense the temperature. In the heating mode, the thermistor is subjected to a 5 s voltage pulse. In the sensing mode, it is part of a Wheatstone's bridge, the output being proportional to temperature. The signal, \( V_i - V_f \), which is the net change in the bridge output from immediately before and after the heat pulse, depends both on the flow rate and the surrounding temperature. Since \( V_i \) is an implicit function of the ambient temperature, a single equation involving both \( V \) and \( V_i \) gives a good prediction for the flow rate, with 6.3% RMS relative error. The sensor behavior was measured for flow rates between 0-52.5 mL/h at 32-39 °C, adequately covering the range of interest. The functionality of flow monitor has been verified on a healthy pig model. Six of eight tests with experimental pigs were successfully conducted with > 4300 acceptable flow measurements. Very good correlation was observed between measured and predicted flow rates (r > 0.9). The average slope of measured vs predicted flow rates was 1.04 ± 0.15, indicating that the flow monitor accurately measured porcine CSF flow.

This work was supported by the Texas Medical Research Consortium.
High-resolution melting (HRM) analysis is a close-tube method for single nucleotide polymorphism (SNP) detection and has shown many advantages in clinical laboratory, point-of-care diagnostics, and personalized medicine. Recently developed melting probes have significantly improved the discrimination of mismatched (mutant) alleles from matched (wild-type) alleles. We, in contrast, aim to design a simple probe that can reliably distinguish mismatched alleles among themselves. Here we demonstrate that a melting probe incorporated with a locked nucleic acid (LNA) thymidine monomer (tL) can reliably differentiate the four SNP alleles by four distinct melting temperatures (termed the “4Tm probe”). This enhanced discriminatory power comes from the decreased melting temperature of the tL·C mismatched hybrid as compared to that of the t·C mismatched hybrid, while the melting temperatures of the tL-A, tL·G and tL·T hybrids are increased or remain unchanged as compared to those of their canonical counterparts. This phenomenon is observed not only in the HRM experiments but also in the molecular dynamics simulations. A possible mechanism is that the tL in the 4Tm probe limits the sugar puckering flexibility and further restricts the ability of the base to form energetically favorable interactions for the tL·C mismatch pair. To our best knowledge, our tL-containing probe is the first demonstration of a working 4Tm probe. Our findings are not only important to the HRM community but also important to the DNA origami and DNA-metal interaction communities as researchers in these two communities are always searching for new ways to fine-tune the affinity between strands and adjust the nucleobase environment around DNA-bound metal atoms.

Reference:

Keywords: Bioanalytical, Biomedical, Biosensors, Nucleic Acids
Application Code: Biomedical
Methodology Code: Sensors
Herein, we developed molecular imprinted polymer recognition layers combined with graphene oxide for cholesterol sensing. Cholesterol-imprinted polymer matrix was prepared by azoisobutynitrile initiated free-radical polymerization of methacrylic acid co-polymerized with ethylene glycol dimethacrylate. Graphene oxide was synthesized by Hummer’s method and combined with cholesterol-imprinted polymer matrix prior to gel formation stage thus, ensuring its uniform dispersion. The non-imprinted polymer layer along with graphene oxide was taken as control to compensate non-specific binding responses. Surface characterization of sensor layers was carried out by atomic force microscopy. The resultant matrix was spin coated to produce thin layer on comb shaped interdigital electrodes fabricated as transducer. The shift in capacitive values of recognition layer was monitored by exposing it against standard cholesterol solutions. The developed sensor setup exhibited remarkably high sensitivity e.g. can recognize cholesterol down to parts per billion level. In selectivity evaluation studies, it was noticed that developed sensor exhibited three fold higher shifts for cholesterol as compare to ascorbic acid and glucose. This suggests that the combination of graphene oxide with molecular imprinted layer results into efficient recognition layers that offer highly sensitive and selective cholesterol sensing.

Keywords: Bioanalytical, Clinical/Toxicology, Material Science, Sensors
Application Code: Clinical/Toxicology
Methodology Code: Sensors
For over 40 years, field effect transistor (FET) based electrochemical sensors have been extensively used in many sensing applications. Recently, a bipolar junction transistor (BJT) based electrochemical sensor is demonstrated to have notably enhanced sensing characteristics in comparison to the widely used FET sensors. Unlike the FET sensor, the BJT sensor has a simpler calibration with no trade-off between sensitivity and resolution, and is particularly well suited for mobile (hand-held or wearable) applications. In this study, two main results are presented. (i) A BJT based pH sensor, comprised of a commercially available discrete BJT device as the transducer and TiN as the pH sensitive sensing surface is evaluated. The BJT sensor has sensitivity and signal to noise ratio that are constant over the entire sensing range. Also, the pH calibration curve is independent of the use voltage. Consequently, the BJT sensor can be calibrated by a single point measurement. This is a significant advantage over the FET sensor that has a complex calibration requirements, where a trade-off has to be made between sensitivity and resolution. (ii) The evaluated BJT sensor is integrated with an automation circuitry and a hand-held pH sensing prototype that displays real time data on a smart phone is demonstrated. The prototype is shown to have high sensitivity, low noise and drift. In summary, sensing results for an automated BJT based electrochemical sensor prototype is presented.

Reference:
Iterative 3D Printed Cast-shrinkage Cycles – A New Route for Prototyping Transdermal Biomedical Measurement Devices

Penetrative needle electrodes have a historic use in electromyography (EMG) when investigating neuromuscular conditions. Surface EMG is a popular, painless, alternative to using needle electrodes that can record muscular activity without having to penetrate into the muscle. Additional impedances from the skin-electrode interface and subcutaneous tissue between the muscle and electrode make surface EMG susceptible to noise. While this can be removed in post processing, there is a serious risk of distorting delicate information in the underlying signal.

Transdermal devices penetrate the first layer of skin making contact with the conductive viable-epidermis (~200 um thick) without penetrating the dermis, containing pain receptors and blood vessels. Transdermal electrodes therefore have lower impedance than surface electrodes. These devices have many potential applications beyond electrophysiology. Transdermal structures have been reported for use in drug delivery [1], vaccinations [2], and biomolecular monitoring[3]. Current manufacture of such devices can be expensive and time consuming, often requiring specialist equipment and materials.

The system uses a polyurethane-water mixture to take a cast of a 3D-printed master, which isometrically shrinks to half its size after solvent evaporation. This process is iterated, using PDMS to form increasingly shrunken replicas of the original master [4]. We have used this process to produce 200 um high transdermal devices, from a 1 mm original. This approach provides a low cost means of improving the resolution of existing 3D-printing/manufacturing devices (reported 4um resolution) [4]. An overview of the process will be given together with examples of a range of biomedical measurement devices.


Keywords: Bioanalytical, Biomedical, Instrumentation, Sensors
Application Code: Bioanalytical
Methodology Code: Sensors
A low-cost optical cavity biosensor using a scaled differential detection method has been proposed for point-of-care diagnostics. In this presentation, we will present the experimental results of biotinylated BSA detection using Streptavidin-Biotin affinity to demonstrate the biosensing capability of the proposed device. The optical cavity structure is designed using a commercial simulation tool, FIMMWAVE/FIMMPROP. To fabricate optical cavity biosensor, we sputter-coated thin silver films on two glass substrates as partially reflective layers. Then, spin-on-glass (SOG) layers are spin-coated onto the silver surfaces. Both SOG layers are coated with 5% solution of 3-aminopropyltriethoxysilane (APTES). One of APTES coated substrate is then coated with 1% bovine serum albumin (BSA) solution to prevent non-specific binding. Then, two substrates are bonded together to form an optical cavity structure which also serves as a microfluidic channel. Streptavidin is introduced into the optical cavity and incubated for an hour to let it bind on one surface. Finally, biotinylated BSA is introduced to be immobilized on streptavidin layer. Each step of the surface immobilization procedure is demonstrated by using fluorescent tagged streptavidin and biotin with a fluorescence microscope. Our low-cost optical cavity biosensor employs two low cost laser diodes. Collimated light waves at the wavelengths of 780 nm and 850 nm propagate through the fabricated optical cavity sample and reach to a CMOS camera to be measured their intensities. As biotinylated BSA binds to streptavidin, intensities of 780 nm and 850 nm change in opposite directions. The differential value is then calculated using them to achieve a larger responsivity. The measurement results will be analyzed and compared with simulation results. This work was supported by NSF grant CBET-1706472.
Graphene nanodisks with good conductivity and plenty of edge sites were synthesized to load glucose oxidase (GRD-GOD) and coupled with a Mn2+ doped CdS QD modified TiO2 electrode (CdS:Mn/TiO2) for highly sensitive photoelectrochemical (PEC) immunoassays. The specific immune-recognition behavior can bring the GRD-GOD labeled antigen into the antibody immobilized CdS:Mn/TiO2 interface and dramatically enhanced the photocurrent response via a dual signal amplification strategy. First, graphene nanodisks with strong electron transfer capacity can improve the conductivity of both the insulating protein layers and the CdS:Mn/TiO2 matrix, thus facilitate the regeneration of trapped carriers and hot electrons in the CdS:Mn QD films and enhance the PEC performance. Second, graphene nanodisks introduce a great number of GOD molecules in a PEC detection process, which can catalyze glucose to produce numerous H2O2. The latter can act as a sacrificial electron donor to scavenge the photogenerated holes, retard the electron-hole recombination, and significantly improve the photo-to-electron conversion efficiency. Based on the dual signal amplification strategy and using carcinoembryonic antigen as a model target, a highly sensitive PEC immunoassay was therefore developed with an extremely low limit of detection of 5.65 fg/mL and a rather wide linear range from 10 fg/mL to 1 ng/mL. The immunoassay also showed good reproducibility and stability, as well as good selectivity and high accuracy in serum sample analysis. Regarding these, our PEC immunosensors may have great application potentials for the screening of tumor markers and the prevention and monitor of serious diseases.
Vibrational Spectroscopy

Validation of ATR Correction and Reverse ATR Correction Algorithms, Improved by Optimized Corrections

To address wavelength-dependent differences in intensity between ATR and transmission FTIR spectra, a mathematical ATR correction can be applied to an ATR spectrum to provide a better match when searching against a transmission FTIR reference database. Conversely, the reverse ATR correction can also be applied mathematically to a transmission FTIR spectrum to provide a better match when searching against an ATR reference database. An original study validated the ATR correction and the reverse ATR correction algorithm in Bio-Rad’s KnowItAll Informatics System spectroscopy software.

This study updates and compares search results from the original study to search results using a patent pending Optimized Corrections technology recently added to the software that iteratively optimizes the query and reference spectra for each comparison by automatically applying multiple corrections to compensate for differences between spectra caused by the variability of different instruments and accessories as well as other factors, including human error. This technology solves many of the issues surrounding spectral search that cannot be adequately addressed by traditional algorithms and manual methods. Corrections include: baseline correction, clipping, horizontal shift, vertical shift, intensity distortion, and ATR correction.

Keywords: FTIR, Infrared and Raman, Laboratory Informatics, Spectroscopy
Application Code: Laboratory Management
Methodology Code: Vibrational Spectroscopy
The Raman spectra of a particular face of a single crystal can be significantly different if acquired with different microscope objectives. The purpose of this presentation is to inform and educate users of micro-Raman instrumentation of the effect of the microscope objective on the Raman spectra of crystals. Furthermore, we explain the underlying physics of changes in relative intensity and even peak position of certain Raman bands depending upon the microscope objective used to acquire the spectrum. Changes in peak position are attributed to phonon directional dispersion sampled through wide angle microscope objectives with different numerical apertures.
Proteins containing expanded polyglutamine (polyQ) tracts aggregate into amyloid fibrils. This fibrillization is associated with at least 10 neurodegenerative diseases including Huntington’s disease. It is thought that strong glutamine side chain-side chain hydrogen bonding interactions play a large role in driving fibrillization and stabilizing the polyQ fibril structure. However, few experimental studies have investigated this. Here we develop a quantitative correlation relating the frequency of the side chain Amide I band to the interaction enthalpy ($\delta H_{int}$) of the primary amide carbonyl group and its environment. This correlation and molecular dynamics simulations are used to estimate the hydrogen bonding interaction strength of side chain carbonyls of the polyQ peptide $D_2Q_{10}K_2$ ($Q_{10}$) in different monomeric solution and amyloid-like fibril conformations. We find that side chain-side chain and side chain-backbone interactions are stronger than side chain-water and backbone-water interactions in polyQ monomers suggesting that glutamine side chains play a large role in aggregation and fibril formation. We also find that side chain-side chain interactions are stronger than backbone-backbone interactions in the antiparallel $[\beta]$-sheet core of polyQ fibrils suggesting that side chain hydrogen bonding contributes greatly to stabilization of polyQ fibrils. Overall, our work quantitatively and experimentally validates the hypothesis that side chain-side chain hydrogen bonding interactions play an important role in the structure, formation, and stability of polyQ fibrils.

Keywords: Bioanalytical, Biospectroscopy, Raman Spectroscopy
Application Code: Bioanalytical
Methodology Code: Vibrational Spectroscopy
Analyses of Consumer Products

Determination of Odorous Compounds in Leather and Leather Products by GC-MS/MS

Introduction:
As the health of human beings was seriously affected by the poisonous and harmful odorous substances remaining in leather and leather products through skin touch or respiratory system, the odorous substances detection in leather and leather products became a social concerned problem. However, it was difficult to detect odorous substances by using common methods for experimenter without odorous substances analysis experience. Therefore, it was urgent to have a rapid screening method for detection odorous substances in leather and leather products. This paper was aimed to establish a rapid and accurate method for odorous components detection in leather and leather products by GC-MS/MS coupled with AOC-6000 auto injection system, and with off-flavor Smart Database.

Method:
A method of 150 kinds of widespread odorous components was found using Off-flavor SmartDatabase which included several parameters of retention index and MRM ions and sense information of these components. The retention time of 150 kinds of odorous substances was calculated by retention index of odorous substances and the retention time and retention index of n-alkanes acquired by analyzing the n-alkanes mix standard. through 3 kinds of internal standards (4-bromofluorobenzene, 1,2-dichlorobenzene-d4, and acenaphthene-d10) analysis acquired the inside calibration curves of 150 kinds of odorous substances, it was easy to achieve semi-quantitative analysis result of 150 kinds of odorous substances in leather or leather product samples. The odorous substance was found by comparing the concentration in sample with the odor threshold registered in the off-flavor database.

Results and conclusions:
A total of 8 samples were analyzed. The result showed that different odorous substances and different contribution were found in all samples due to different source and manufacture. this method was quick, simple and useful for odorous substances analysis in leather and leather product.

Keywords: Contamination, Database, Gas Chromatography/Mass Spectrometry, SPME

Application Code: Consumer Products

Methodology Code: Gas Chromatography/Mass Spectrometry
Pyrolysis-GC/MS is a technique which allows for the analysis of organic materials which are too large to be compatible with gas chromatography. Material is heated in a controlled way, and large molecules, often polymers, are broken into smaller, volatile compounds which can be analyzed by GC and mass spectrometry; specific information on the attributes of the original material is conserved. This way, analytical pyrolysis produces results which are polymer specific, making it the perfect tool for analyzing all types of organic polymers, including natural textiles, such as leather.

True leather is made from animal skin, often cattle hide. It undergoes chemical treatment, tanning, to alter the protein structure, making it more durable and less susceptible to decay. Other finishes, such as dyes, or coatings to provide other characteristics such as water-fastness or a shiny appearance may also be applied. Pyrolysis GC/MS will be used to investigate leather like textiles including imitation real leather, and different forms of leather for similarities, differences and additives including dyes, tanning and finishing agents.

**Abstract Text**

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**Keywords:**  Consumer Products, Gas Chromatography/Mass Spectrometry, Pyrolysis, Sample Introduction

**Application Code:**  Consumer Products

**Methodology Code:**  Gas Chromatography/Mass Spectrometry
Electronic cigarettes (e-cigs) began as an alternative to smoking a traditional cigarette but have rapidly become a way of consuming drugs other than nicotine (DOTNs). The addition of alkaloid-containing, plant-based products are emerging and advertised as “all natural” that produce a range of effects that are stimulating, euphoric, and even “a substitute for opium”. The objective of this research is to analyze e-liquids and their respective resins and powders for the pharmacologically active alkaloids that can be attributed to producing the marketed effects by Jeol JMS T100LC AccuTOF DART-MS. E-liquids were also submitted to volatile organic compounds analysis by headspace gas chromatography with a flame ionization detector (HS-GC-FID).

Eight e-liquids, five resins, and four powders purchased from Lotus Extracts and an e-liquid purchased from Bizarro were analyzed and as a 1:10 dilution by DART-MS. The data was analyzed using T.S.S Pro version 3.0 and other potential compounds were detected using a NIST 11.0 library. Volatiles were analyzed by HS-GC-FID using a Restek BAC-2 column.

The e-liquid products' ingredient labels only identified the plant product, propylene glycol, and vegetable glycerin. After presumptive analysis of the various samples, the compounds that were consistently detected included arecoline, arecaidine, apomorphine, nuciferine, mitragynine, 7-hydroxymitragynine, coumarin, PG, and VG. Volatile analysis by HS-GC-FID resulted in identification and quantitation of isopropanol and ethanol in five products. This study demonstrates the evolving industry for products containing psychoactive substances to be used in e-cigs. Public misconception that all “natural” products are healthy/safe contribute to risk of public health, especially given the general lack of information on ingredient labels on these products. This research demonstrates the presence of psychoactive and potentially harmful substances are present in e-liquids intended to be vaped.

Keywords: Consumer Products, Forensic Chemistry, Gas Chromatography, Time of Flight MS
Application Code: Consumer Products
Methodology Code: Mass Spectrometry
Thermal property characterization of food and cosmetic products are complicated due to their heterogeneous nature. They contain most often crystals, emulsion droplets or big particles. Consequently, conventional techniques, such as Differential Scanning Calorimetry (DSC) are often limited by the small non-representative quantity of sample, which can also induce denaturation of the product.

Recently, Diffusing Wave Spectroscopy (DWS) has been coupled with an accurate temperature control, to analyze microstructure evolution, of a complex product during heating or cooling. Multi Speckle Diffusing Wave Spectroscopy (MS-DWS) [1, 2, 3], in which the backscattered light of a coherent LASER is analyzed with a temporal resolution, allows a correlation of the particle motion in the sample and the intensity fluctuation of the backscattered light. The combination of this technique with an accurate temperature control provides information about the microstructure evolution during thermal processing. Indeed, during phase transition, the microstructure evolves faster, which can be observed as characteristic peaks. Various number of cosmetic products are based on fatty compounds, such as esthers, waxes, oils, etc. The formulation of these products must be carefully chosen to avoid, exudation, oil segregation or other phenomena of destabilization. Especially, cosmetic products are stressed thermally by important variations of temperature (indoor, outdoor, summer, winter,…), which can provoke quality problems (surface crystallization and/or exudation).

Characterization of crystalline forms and their variation after thermal stress (cycles) can be performed with RHEOLASER Crystal® and can improve significantly the formulation process.

### Session Title
Chemical Methods

### Abstract Title
The “Priming” Effect: Previous Exposures and Activities May Impact Current Responses to Environmental Stressors in Certain Individuals

### Primary Author
Joachim D. Pleil
US EPA

### Co-Author(s)
Ariel Wallace, Michael C. Madden

### Abstract Text
In recent public health studies, researchers have found that there is a large variability in biomarker response to similar environmental exposure conditions. In the broadest sense, this is a result of the “gene x environment” interaction wherein the outcomes are dictated by a complex synergy of the human exposome and genome. One part of the explanation for variable outcomes may be that a previous day’s exposure has somehow modified the human systems biology to be more or less susceptible to today’s exposure. Termed the “priming” effect, this was studied in controlled experiments using typical urban environmental exposure followed after 24-hrs by an ozone challenge. The results were monitored by pulmonary function tests in the form of forced expiratory volume (FEV1) and measures of inflammatory cytokines in exhaled breath condensate (EBC). Changes in FEV1 were correlated with differential cytokine expression for a subset of individuals, and totally uncorrelated for others. This work suggests that previous activity should be considered in observational studies of public health environmental epidemiology.

### Keywords:
Bioanalytical, Biological Samples, Environmental/Biological Samples, Immunoassay

### Application Code:
Environmental

### Methodology Code:
Chemical Methods
Ionic liquids (ILs) have received much attention during the past few decades due to their unique chemical and physical properties. They are often quoted as having low vapor pressures, low flammabilities, and they are thermally and chemically stable. Therefore, they are widely used in various pharmaceutical and industrial applications, but despite their “green reputation” the environmentally unfriendly features of ILs have been under a lot of investigation recently. The toxicological risk abides throughout the whole IL life cycle, from synthesis to disposal. Hence, it is also essential to acknowledge the potential hazards related to the possible degradation products of the ILs. The underlying mechanism of their toxicity and the physicochemical properties of ILs affecting biomembrane interactions are still largely unknown. In this work, cytotoxicity data was compared with results obtained from analytical physico-chemical techniques using phospholipid vesicles (liposomes) as model biomembranes. The aim was to gain novel information on the underlying mechanism behind the cytotoxicity of ILs.

The cytotoxicities of ILs were assessed using three organisms: Chinese hamster ovary cells, human corneal epithelial cells, and Vibrio fischeri marine bacteria. To investigate the influence of ILs on cell rupturing, hemolysis was performed using human red blood cells, and the results were compared with liposome integrity data. The effect of ILs on the size and the zeta potential of negatively charged liposomes were assessed. In addition, differential scanning calorimetry and pulsed field gradient nuclear magnetic resonance spectroscopy were utilized for studying interactions between the compounds and the liposomes. The result show that the investigated ILs can be divided into three groups based on the cytotoxicity mechanism; cell membrane breaking ILs, partially cell membrane breaking ILs, and ILs that affect the cell metabolism inter- or intracellularly.

Keywords: Bioanalytical, DSC, Environmental/Water, Magnetic Resonance
Application Code: Safety
Methodology Code: Chemical Methods
Chemical Methods

Synthesis of Titanium Oxide Layer on Titanium by Hydrothermal Method

Nowadays various titanium oxide layers are widely used for different applications. Solar cells are one of areas where titanium oxide is widely used. Despite this fact there is still a high interest in synthesis of new titanium oxide layers using different methods for new applications in practice. Properties of titanium oxide and its application areas depends on synthesized layer thickness, composition and surface morphology [1,2]. This study is focused on synthesis and characterization of titanium oxide layers formed on pure titanium foil or titanium layer sputtered on microscopic glass. Hydrothermal synthesis was selected due to simple control of the process. It was observed that many properties of synthesized titanium oxide layers as well as band gap depend on synthesis conditions. Scanning electron microscopy (SEM), X-ray diffraction (XRD) and inductively coupled plasma mass spectrometry were employed in this study for the characterisation of formed layers.

References:

Keywords: Material Science, Surface Analysis, Wet Chemical Methods, X-ray Diffraction

Primary Author
Simonas Ramanavicius
SRI Center for Physical Sciences and Technology

Co-Author(s)
Arunas Jagminas

Material Science, Surface Analysis, Wet Chemical Methods, X-ray Diffraction
Material Science
Chemical Methods
Pyrazolines are very important hetero-atom Nitrogen containing five membered heterocyclic chemical compounds and various methods have been worked out for their synthesis. A new series of 4-(4-Hydroxy phenyl)-3-chloro-1-{4-[5-(Substituted phenyl)-1-acetyl-4,5-dihydro-pyrazol-3-yl]phenyl}azetidin-2-one are synthesized by reacting 3-chloro-1-{4-[5-(Substituted phenyl)-4,5-dihydro-pyrazol-3-yl]phenyl}-4-(4-Hydroxy phenyl)azetidin-2-one (0.001M) with Acetic Acid in presence of Pyridine. All these compounds were characterized by means of their IR, 1H NMR, Spectroscopic data and pharmaceutical analysis. All the compounds were tested for their antibacterial and antifungal Biological activity by broth dilution method.

Keywords: Analysis, Chemical, NMR
Application Code: Pharmaceutical
Methodology Code: Chemical Methods
Arsine (AsH₃) is one of the most dangerous gases commonly found during hydrocarbon extraction operations. This gas monitoring requires a very strict control given the low exposure limits (below 0.05 ppm) established for the Occupational Safety and Health Administration. Besides, the presence of arsine in refineries and gasification processes represents a critical control point in economic terms, and additionally it may contribute as a potential environmental issue. Dry colorimetry offers an analytical solution, at low costs, for arsine quantification at trace levels for laboratory and on line applications with reliable results. The poster presentation would include experimental results for the arsine quantification in gas analysis considering very low concentration ranges (1-50 ppb) probing the efficiency and accuracy of the methodology proposed.

Keywords: Analysis, Calibration, Fuels\Energy\Petrochemical, Petroleum
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Chemical Methods
A Novel Method for Determination of Sucralose in Environmental Samples

Sucralose, a chlorinated synthetic sweetener, is persistent in environmental waters and is considered an emerging contaminant of concern. In this study, we present a novel method for the extraction and derivatization of sucralose in environmental water samples compared to existing analytical methods. Sucralose was extracted from water samples using solid phase extraction and selectively eluted with tert-butyl methyl ether to remove excess impurities. The extract was esterified with benzoyl chloride in acetonitrile to synthesize a UV-active derivative. The reaction produced a higher yield over existing methods for sucralose derivatization. The derivatized sucralose products were analyzed by reversed phase high performance liquid chromatography and detected by UV absorption. Good separation was achieved in a gradient elution with acetonitrile and water in less than 10 minutes. The method presented provides a simple and effective quantification of sucralose in environmental samples with high specificity and without the need for mass spectrometry.

Keywords: Derivatization, Environmental/Water, HPLC, Solid Phase Extraction
Application Code: Environmental
Methodology Code: Chemical Methods
Abstract Text
Most cells in the human body respond to growth hormone, which binds to cell surface receptors and induces growth and replication for those cells. Our study is focusing on receptor tyrosine kinase (RTK), a cell receptor of growth hormone that phosphorylates downstream proteins inside the cells, leading changes in certain protein expression level that allows for cell growth and replication. Utilizing this pathway has become one of the most popular fields in regenerative therapy, however, uncontrollable cell growth would be oncogenic. In our project, we design the DNA reactions to realize light-responsive switch between the activation/deactivation of the RTK-participated signal pathway. 

The activation of RTK pathway requires the close proximity of the two receptor subunits when bound with the growth factor, which would be achieved by using the DNA aptamers of the RTK protein and the formation of the double-stranded structure (Figure 1). The introduction of the PC-linker would allow the feasible cleavage in the DNA structure, causing the disassembly of the receptor pair. The reaction was monitored with FRET and gel-electrophoresis in solution, and flow cytometry on the cell membrane. The phosphorylation of the proteins would be analyzed with western-blotting and other cellular experiments. This design would offer a feasible, fast, and simple platform for controllable cell regeneration.

Keywords: Biomedical, Fluorescence, Nucleic Acids, Protein
Application Code: Biomedical
Methodology Code: Chemical Methods
Often times, refiners cannot predict precisely when coking will occur so they stop processing crudes well short of coke formation. In order to prevent fouling in heavy oil processing equipment, tanks and transfer lines, they stop processing too soon and reduce the distillate yield.

Automated Flocculation Titrimeter (AFT) technique automates the collection of Heithaus values and predicts flocculation for various crudes. It also allows users to easily and reliably predict which heavy oils and petroleum residua can be mixed without causing phase separation.

The theory behind the development of this innovative and advanced technique forms the basis of this poster. Petroleum residua consists of ordered structures of associated polar asphaltene complexes that are dispersed in a lower polarity solvent phase by intermediate polarity material commonly referred to as resins. When the residuum is heated to temperatures above 340 degrees Celsius, this suspended structure is systematically and irreversibly destroyed during pyrolysis. A certain amount of pyrolysis typically occurs and can be tolerated in a distillation unit since there is an induction period prior to the appearance of coke. A common problem in the refining industry is to ascertain how close a pyrolysis system is to forming coke on the coke induction period timeline.

The automated flocculation titrimeter has been developed to perform ASTM D6703, the official test method for automated Heithaus titrimetry. This helps to measure the state of the dispersed particle system and calculates predictive parameters for heavy oils. The advanced AFT can now be used to provide valuable information about the internal stability of a heavy oil, the proximity of a pyrolyzed oil to coke formation and to design blending protocols for oils mixtures related to prevent asphaltene precipitation, as well as study flocculation kinetics. It is a versatile tool for the petroleum industry in both upstream and downstream operations.
Molecular imprinted polymers (MIP) may in principle be used as alternatives to antibodies for recognising proteins. However it is more difficult to imprint proteins than small molecules e.g. because of the flexible conformational changes of proteins. However with ‘surface-etching’ it should be possible to produce a recognition site on a support material without compressing and distorting the protein tertiary structure. So, the aim of this study was to find if a surface ‘etch’ could be used to create materials which interacted selectively with the protein that had been used for etching. Bovine serum albumin (BSA) was used as a model protein and surface-etched silica sub-micron particles for BSA were prepared. With magnetic versions of such materials it was possible to demonstrate that BSA could be selectively adsorbed in the presence of a range of potentially interfering proteins and to study the effect of experimental variables on selectivity. Importantly, it was confirmed that coating agents were retained on the particle after digestion of the etching protein. Thereafter further studies on potential influences such as isoelectric point, pH, type of competing protein, size of competing protein, cross-reactivity, adsorption kinetics and temperature were carried out and building on this it was demonstrated that the particles could be deployed in an immunoassay format. Additionally, etched particles for other proteins were prepared and evaluated. The final phase of the programme involved attempts to put etched-protein particles to good use e.g. etched protein particles for human chorionic gonadotropin (hCG), of potential commercial value in the context of a non-antibody based diagnostic kit were successfully prepared. These exhibited good selectivity towards hCG which was unaffected by the presence of other proteins. Overall, the development, validation and application studies carried out suggested good promise for this type of highly selective binding agent.
Antimicrobial resistance is a global concern that affects to more than 2 million of people each year. Therefore, new approaches to kill bacteria are needed. One of the most promising methodologies may come from metallic nanoparticles since bacteria cannot develop resistance to these nanostructures. While metallic nanoparticle synthesis methods are well studied, they are often accompanied by significant drawbacks such as cost, extreme processing conditions, and toxic waste production. In this work, we explored the environmentally safe synthesis selenium nanoparticles, which have shown promise in killing bacteria.

Using Escherichia coli (strain K-12 HB101; Bio-Rad, Hercules, CA), Pseudomonas aeruginosa (Schroeter, Migula, ATCC® 27853™), Staphylococcus aureus subsp. aureus Rosenbach (ATCC® 12600™) and Methicillin-resistant Staphylococcus aureus (MRSA) (F-182, ATCC® 43300™) were used to synthesize biogenic selenium nanoparticles, 90-150 nm selenium nanoparticles were synthesized under standard conditions using an environmentally-safe approach. The nanostructures were characterized using Transmission Electron Microscopy (TEM, JEOL USA Inc., MA) and Energy Dispersive X-Ray (EDX) to determine the chemical composition. Nanoparticles were also characterized and tested for their ability to inhibit the bacterial growth. Besides, biocompatibility tests of the nanoparticles with human tissue were accomplished, growing human dermal fibroblast (HDF) cells.

Therefore, it is demonstrated that the bacterial strains used can generate biogenic selenium nanoparticles. with antimicrobial activity against both Gram positive and Gram negative bacteria. In vitro cytotoxicity assays were performed with human dermal fibroblasts (HDF) cells. The experiments showed that all nanoparticle concentrations tested did not inhibit the growth of cells while also confirming a high percentage of cell viability.

Keywords: Biological Samples, Biomedical, Environmental, Nanotechnology
Application Code: Biomedical
Methodology Code: Chemical Methods
Targeted Gene Editing by Aptamer-CRISPR/Cas9 Bioconjugates

CRISPR/Cas9 has recently become the most developed and utilized gene editing technology in many academic fields, including biology, genetics and cancer research, due to its simplicity, versatility and specificity. Double-strand DNA breaks introduced by CRISPR/Cas9 afford researchers the opportunity to either change target the gene by precise modification or to use non-template repair with high genetic mutation rate to make animal models. However, this inevitably elicits two main issues: for one thing, off-target effects of CRISPR/Cas9 system may lead to irreversible adverse consequences with a non-template repair pathway, and second, precise modification of genes is not accessible in many cells. Targeted delivery of CRISPR/Cas9 system should be one of the solutions to minimize these problems since fewer CRISPR/Cas9 will be employed to achieve the therapeutic effects. Therefore, we hypothesized that bioconjugates of NHS ester-modified aptamer with lysine residues in Cas9 should be actively delivered to the target cells via cell targeting ligand-aptamer. The resulting bioconjugates will be assessed in terms of bioactivity, binding affinity, internalization and in vitro gene editing efficiency.

Keywords: Biomedical, Chemical, Protein
Application Code: Biomedical
Methodology Code: Chemical Methods
Series of complexes with general composition \([ML2(H2O)2](where M = \text{Co(II)}, \text{Ni(II)}, \text{Cu(II)})\) have been prepared. The thiosemicarbazone ligand and metal complex have been characterized by elemental analysis, FT-IR, NMR, magnetic measurement, specific conductivity measurement, electronic spectra, thermal studies, and biological activity. The present ligand acts as a monobasic bidentate ligand and coordinates to the metal ions through thiolate sulfa and azomethene nitrogen. Thermal decomposition of the metal complexes indicate loss of water of hydration and decomposition of the ligand. The complexes of transition and non-transition metals with thiosemicarbzones have been studied extensively. Thiosemicarbazone have gained special attention due to their activity against protozoa, influenza, small pcox, malaria, tuberculosis, antimicrobial and antitumor activity of their metal complexes. Metal complexes of thiosemicarbazones have emerged as a new class of chemotherapeutic agents which exhibit inhibitory activity against most cancers through inhibition of a crucial enzyme. Owing to the wide range of medicinal use of metal complexes of thiosemicarbazone and in continuation of earlier work on metal chelates of thiosemicarbazone. Several pyrazoline are also useful as biodegradable agrocheeds and intermediates in dye industry. Electronic spectra and magnetic susceptibility measurements reveal octahedral geometry around the metal ion.
Quantitative Analysis of Iron Suspended in Silicone-Based Fluids and Gels

Silicone-based fluids and gels find wide commercial application in the production of sealants, elastomers, adhesives, hydraulic fluids, and in food packaging and processing. In most applications, the silicone fluid is added in order to take advantage of its fundamental physical and chemical properties but does not typically play an active role. The addition of magnetic particles, such as magnetite (Fe$_2$O$_3$), as a suspension of nanoparticles imparts control functionality allowing for pressure and flow management, focused localization, and variable extrusion behavior. The magnetite particles are typically synthesized in an aqueous medium followed by extraction into a small volume of silicone-based fluid, which sometimes is further modified by the addition of a copolymer with chemical, thermal or radiative cross-functionalization. This method of embedding the nanoparticles in the silicone fluid is quantitatively imprecise. Unfortunately accurate determination of the concentration of magnetite in silicone has historically been challenging due to the incompatibility of silicone materials with standard atomic methods and the solvents used therein. We have developed a straightforward protocol for the quantitative determination of iron in silicone-based fluids and polymers that is amenable to inexpensive atomic spectroscopies. The method is fundamentally based on dispersion in an organic solvent followed by acid digestion and extraction of the dissolved iron into aqueous solution prior to introduction into a flame atomic absorption spectrophotometer. We will present the results of our analyses of both silicone-based fluids and silicone-based polymer gels.

Keywords: Atomic Spectroscopy, Dissolution, Method Development, Sample Preparation
Application Code: General Interest
Methodology Code: Chemical Methods
A series of perylene tetracarboxylic diester monoanhydrides (PEAs) which include labile functional groups in their flexible side chains synthesized via intermolecular cyclization. Those structures were not accessible through the previously reported method. Moreover, using alcohol at the first step in the presence of 1,8-Diazabicycloundec-7-ene enables the synthesis of unsymmetrically substituted PEAs. As PEAs are capable of forming bundled-stack discotic columnar liquid crystalline (BSDCLC) phase is attribute to the unique perylene anhydride inter-stack interaction, availability of the procedure reported here would enable the integration of various side chains onto the PEA aromatic core which is crucial to optimizing and studying organic charge transport materials with BSDCLC phases.

Keywords: Liquid Crystal, Semiconductor, X-ray Diffraction
Application Code: Material Science
Methodology Code: Chemical Methods
A series of optical active pyrano[3,2-c]chromenes have been synthesized through dehydroabietylamine-cinchonine-squaramide catalyzed asymmetric domino reaction of malononitriles with 4-hydroxy-2H-chromen-2-ones. The targeted molecules were obtained in excellent yields and enantioselectivities (up to 94% yield, 99% ee). With this asymmetric organocatalysis methodology in hand, we have prepared variety chiral compounds with (R)- or (S)-configuration, and the inhibitory activities of these chiral heterocyclic compounds have been compared. The preliminary biological studies revealed several compounds showed potent anti-AChE activity, in which compounds 4n (IC50 = 21.3 [micro]M) and 4p (IC50 = 19.2 [micro]M) displayed potent acetylcholinesterase inhibition. In most cases, the S-enantiomers were superior to the corresponding R-enantiomers. Moreover, the molecular modelling provides practical way for understanding the enantioselective discrimination of AChE with this kind of compounds.
**Abstract Title**: Rapid Analysis of PFCs in Non-drinking Water Matrices

**Primary Author**: Brahms Prakash

**Shimadzu Scientific**

**Co-Author(s)**: William Lipps

**Abstract Text**

Measurement of Perfluorinated compounds is often done after solid phase extraction, concentration of the sample extract, and injection into an LCMSMS. Newer LCMSMS instruments are sensitive enough that the sample plus methanol can be directly injected into the instrument. ASTM D7979 samples small volumes of sample adding methanol directly into the sample vial eliminating loss of sample to the container walls. This presentation covers our experiments with ASTM D7979 including long-term stability, peak shape, chromatography optimization, detection limits and precision.

**Keywords**: Environmental, Environmental/Waste/Sludge, Environmental/Water, Method Development

**Application Code**: Environmental

**Methodology Code**: Chemical Methods
Retardation of the Phase Segregation Process of Liquid Mixtures with a Critical Point of Miscibility

The Phase Transition Extraction (PTE) process consists of bringing a partially miscible mixture from the single-phase to the two-phase region of its phase diagram by changing either its temperature or its concentration. Its most important characteristics is that the resulting phase separation is very fast. In this work we study two counter-cases, where the PTE process appears to be severely retarded. First, we consider the ouzo effect, where a mixture of water, acetonitrile, and toluene phase separates by homogeneous nucleation, forming very stable micro-emulsions. The second retardation technique consists of using very viscous solvents. We found that the growth rate of the nuclei during the initial stage of phase separation does not depend on viscosity, while, on the other hand, when the size of the nuclei exceeds their capillary length, gravitational effects become relevant and the settling time is proportional to the viscosity of the continuous phase.

Keywords: Extraction
Application Code: General Interest
Methodology Code: Chemical Methods
Precise and accurate results of water contents are important for the quality of raw materials used in the production process and as well for the finished products. Whether you are determining the water content using the volumetric Karl Fischer titration or using a coulometric Karl Fischer instrument there are many aspects of the applied measurement method which require careful consideration to ensure that the results measured are accurately giving the water content in the sample. You will learn to fulfil compliance requirements and to develop your application to produce reproducible and comparable results. A correct pre-titration to eliminate the water in the titration cell and in the solvent as well as a precise titer determination or instrument check is an important precondition. How you can detect and avoid influences on your titration results will be discussed. With appropriate measures, you will be well prepared for quality assurance requirements and for internal and external audits. A further challenge is the individual development of the right application for each sample. An important aspect is the complete water release. Different techniques will be discussed. Dissolving the sample directly in the Karl Fischer solvent or do an internal or external extraction depends always upon the property of each sample. Additional solvents can improve the solubility of the sample. Heating up the medium or a dispersion can also support the water release. Disturbing side reaction can lead to false results and need to be recognized and suppressed. Each application need to be adapted individually to the sample with special techniques or reagents. We shall speak about how to develop the application, standardize the system and how to be prepared for the next audit.
Particulate Matter (PM) air pollution is known to cause negative human health effects by causing oxidative stress from reactive oxygen species (ROS) generation. The dithiothreitol (DTT) assay is popular in measuring oxidative load of PM and correlates with PM causing cellular damage. To correlate to ROS activity, DTT concentration can be measured using spectroscopic and/or electrochemical methods. Electrochemical detection is advantageous because it eliminates dilution factors from added chromophore reagents, as well as requiring fewer steps and less time per measurement. However, current electrochemical protocols use non-automated formats, which can be laborious due to the need for repeated calibration with the other assay requirements. In this work, we have developed a system that combines a wall-jet electrochemical cell with an autosampler towards a high-throughput, automated DTT assay. The cell is fabricated from thermoplastic carbon electrodes (TPEs). TPEs have excellent electrochemical performance (conductivity up to 1,000 S/m) while remaining low cost (< $1/device). Due to the low sample values required (20 [micro]L), as well as the miniaturized electrochemical system, data points could be collected at an unprecedented interval of 1 point every two minutes. The wall-jet cell has coulometric efficiencies approaching 100%, eliminating the need for calibration. Finally, as a proof of utility, filter samples from a cookstove replacement program in Honduras were analyzed for their PM oxidative toxicity. Excellent correlation between the high throughput electrochemical assay and traditional assays were found.

Keywords: Aerosols/Particulates, Calibration, Sample Handling/Automation, Sensors
Application Code: Environmental
Methodology Code: Electrochemistry
Amorphous carbon thin films eject electrons upon irradiation with UV light, making them potential candidates as a photo-catalytic interface for redox reactions, such as generating CO from CO2. In order to attach redox active complexes to amorphous carbon thin films, stable chemical attachment strategies must be developed. Recently, we modified amorphous carbon thin films with thiols to perform photo-initiated thiol-ene click chemistry. While we were able to selectively photo-pattern these films, the thiol-terminated surfaces readily oxidized. In order to circumvent this oxidation issue, we have investigated modifying amorphous carbon films with Grignard reagents to yield vinyl terminated surfaces. Directly attaching vinyl groups to the amorphous carbon films affords three distinct benefits: a chemical handle to attach redox active complexes, a tunable work-function for photo-catalytic reactions, and a stable interface that is less prone to oxidation than the thiol-terminated film. To confirm the utility of the vinyl group to serve as a way to attach redox-active complexes, photo-initiated thiol-ene click chemistry was performed. Upon successful modification of the thin film with a redox active complex, we monitored the photocurrents produced upon illumination of the film with UV light. In summary, this work focuses on strategies for the attachment of redox-active complexes and their use as a photo-catalytic interface for surface bound redox reactions. To the best of our knowledge, these are the first studies to evaluate amorphous carbon thin films as a photo-catalytic interface.

**Keywords:** Chemically Modified Electrodes, Electrochemistry, Spectroscopy, Surface Analysis, Chemically Modified Electrodes, Energy and Petrochemical

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

**Methodology Code:** Electrochemistry
Electrochemical Sensing

Evaluating the Photo-Catalytic Properties of Chemically Modified Amorphous Carbon Electrodes

Amorphous carbon thin films eject electrons upon irradiation with UV light, making them potential candidates as a photo-catalytic interface for redox reactions, such as generating CO from CO2. In order to attach redox active complexes to amorphous carbon thin films, stable chemical attachment strategies must be developed. Recently, we modified amorphous carbon thin films with thiols to perform photo-initiated thiol-ene click chemistry. While we were able to selectively photo-pattern these films, the thiol-terminated surfaces readily oxidized. In order to circumvent this oxidation issue, we have investigated modifying amorphous carbon films with Grignard reagents to yield vinyl terminated surfaces. Directly attaching vinyl groups to the amorphous carbon films affords three distinct benefits: a chemical handle to attach redox active complexes, a tunable work-function for photo-catalytic reactions, and a stable interface that is less prone to oxidation than the thiol-terminated film. To confirm the utility of the vinyl group to serve as a way to attach redox-active complexes, photo-initiated thiol-ene click chemistry was performed. Upon successful modification of the thin film with a redox active complex, we monitored the photocurrents produced upon illumination of the film with UV light. In summary, this work focuses on strategies for the attachment of redox-active complexes and their use as a photo-catalytic interface for surface bound redox reactions. To the best of our knowledge, these are the first studies to evaluate amorphous carbon thin films as a photo-catalytic interface.
The oxygen reduction reaction (ORR) is one of the most studied reactions in the field of electrocatalysis, partly because of its importance to the commercialization of polymer electrolyte membrane fuel cells (PEMFC). Platinum, as a cathode electrocatalyst, offers stable over-potential characteristics and relatively fast kinetics for the ORR when used in acidic media. However, the rarity of Pt drives the need for alternative PEMFC cathode materials. While a variety of non-platinum materials have been investigated for this purpose, none have reached the commercialization stage. For the continuing development of electrocatalyst materials, experimental methods are needed that can obtain accurate kinetic data alongside mechanistic information that gives insight into the ORR pathway. This contribution describes an RRDE setup that can be used to characterize and evaluate ORR electrocatalysts and presents example data using Pt.
The electrochemical reduction of molecular oxygen (ORR) imposes a key bottleneck to the development and widespread implementation of low temperature fuel cells and metal-air batteries: due to the lack of suitable catalyst, sluggish kinetics, and high overpotentials. This has resulted in significant research efforts to better understand the underlying mechanism of ORR, as well as the production of numerous ORR catalyst materials over the past several decades. However, methods capable of rapidly interrogating the underlying structure-activity relationships of electrocatalyst surfaces are limited in number and capability. Furthermore, ORR activity at polycrystalline, nanoparticle, and single-crystal electrodes have shown that their structure significantly effects the ORR kinetics and mechanism. This leads to difficulties in determining active sites at most ORR catalysts due to their heterogeneity: comprised high-index surfaces, kinks, atomic steps, and grain boundaries.

Here, we show the correlation between electrode surface structures at pseudo-single-crystal Au electrodes and their activity for ORR in alkaline media. Galvanically etching a polycrystalline Au electrode exposes numerous well-defined crystallites that can serve as pseudo-single-crystal electrodes. Scanning electrochemical microscopy (SECM) combined with electron backscatter diffraction (EBSD) was used to elucidate the crystallographic orientation dependence of Au on its ORR activity. Tip generation substrate collection (TGSC) SECM produced electrochemical activity maps of the entire Au surface (i.e. crystallographic orientations and boundaries). This method promises a facile and rapid approach for analysis of surface activity relationships of ORR catalysts.


Keywords: Electrochemistry, Electrode Surfaces, Imaging, Microelectrode
Application Code: Material Science
Methodology Code: Electrochemistry
Electrochemical Sensing

Light Activated Electrochemistry: Towards Electrochemical Mapping of Nanoparticles

Semiconducting electrodes that are in depletion, can support appreciable electron transfer only when illuminated. This phenomenon has been implemented on silicon electrodes passivated by a protective organic self-assembled monolayer (SAM) to confine electrochemistry to microscale regions on an unstructured electrode surface by shining light only on those regions. This method, referred to as light-activated electrochemistry (LAE), has so far only been studied using electrodes with surface-bound redox mediators that are either covalently or electrostatically attached to a SAM-modified silicon electrode. In the current report, we extend LAE to redox species in solution using gold nanoparticles (AuNPs) attached to the SAM-modified silicon electrodes, followed by mapping the position of the NPs on the surface using a modulated state-of-the-art light projector, to selectively address the NPs at a very fast rate.

Abstract Text

Co-Author(s) Justin Gooding

Keywords: Electrode Surfaces, Nanotechnology, Semiconductor
Application Code: Material Science
Methodology Code: Electrochemistry
Herein, we demonstrate a series of water-stable, adaptive, and electroactive supramolecular ionic materials (SIM) that is formed from the aqueous solutions of imidazolium-based dication and dianionic through ionic self-assembly. The formed SIM not only shows good thermostability and unique optical and electrochemical properties that are raised from precursors of the SIM, but also exhibits good water-stability, salt-stability, and adaptive encapsulation properties toward some heterocyclic cationic dye molecules. Firstly, we systematically studied the self-assembly behavior based on different carbon chains in imidazolium dications and its structure. Secondly, we found these SIM bears good adaptive inclusion property towards organic dyes and inorganic salt. Based on this, some kinds of biosensors including fluorescence and electrochemistry have been developed. Finally, the electronic property of this kind of SIM was investigated. And we found its electric conductivity was strongly dependent on the humidity. Based on this property, the solid electronic device with high sensitivity and stability was developed. The present study not only opens a new avenue to the preparation of the supramolecular materials, but also provides a versatile platform for (bio)sensing and electronic devices.

**Keywords:** Bioanalytical, Biosensors, Electrode Surfaces, Monitoring

**Application Code:** Bioanalytical

**Methodology Code:** Electrochemistry
A Closed AC Bipolar Electrode ECL Sensing Platform toward Monolayer Detection

A highly sensitive electrogenerated chemiluminescence (ECL) sensing platform based on a closed bipolar electrode (BPE) configuration has been developed that employs a AC square-wave excitation for signal amplification. The closed BPE cell is comprised of two separate compartments fabricated by photolithography and 3D printing techniques. It allows a wide variety of redox-active analytes to be detected in the sensing compartment without interference from the reporting compartment and ECL background from driving electrode, greatly expanding the applicability of ECL reporting system. Redox-initiated change in the analyte at the cathode of the BPE is directly read out by a photomultiplier tube (PMT) above the anode where ECL is occurring. In contrast to conventional bipolar systems controlled using DC voltages, the AC square-wave technique described herein allows electroactive species to be regenerated by applying a reverse potential during the following half cycle. Significant signal amplification can be achieved by accumulating successive ECL signals over multiple measure-regenerate cycles. Using ferricyanide as a model analyte, the basic working principles of the AC SW-BPE method were demonstrated for diffusion based systems. Surface-confined analyte, was first characterized by electropolymerized methylene (MB) thin films on BPE with sufficient surface concentration. Finally, we demonstrated the ability to detect MB-conjugated DNA assembled monolayer on BPE by multi-cycle signal accumulation with a detection limit of surface coverage < 300 fmoles/cm² on both low and high AC frequency. In addition, frequency dependent ECL emission in this system was investigated via solution potential measurement. This sensing system is extremely flexible, capable of detecting a wide variety of analytes over a broad concentration range, and even amenable for monolayer detection and quantification via ECL readout, showing great promise in numerous analytical applications.

Keywords: Bioanalytical, Chemiluminescence, Electrochemistry, Sensors
Application Code: Bioanalytical
Methodology Code: Electrochemistry
Abstract Text
Kale and Grapes are included in the USDA’s national pesticide residue monitoring program for produce and are in increased demand and consumption globally. The use of a QuEChERS technique followed by LC-MS/MS has proven to be an effective approach for screening multiresidue pesticides from various produce items including kale and grapes. Following the EN 15662 pesticide residue method using roQ™ QuEChERS extraction kit and clean-up dSPE kit, along with the use of a high efficient and wide polarity Kinetex®5 µm Biphenyl Core-Shell HPLC column, a pesticide screening method was developed using LC-MS/MS in under 16 minutes. Over 100 Pesticides were screened at concentration ranges between 0.05 ppm to 1 ppm with a recovery range of 70-130%. The method produced excellent recoveries and selectivity for the earlier eluting polar pesticides owing to the biphenyl phase functionality of the Kinetex Biphenyl column.

Keywords: HPLC, HPLC Columns, Liquid Chromatography/Mass Spectroscopy, Mass Spectrometry
Application Code: Food Safety
Methodology Code: Liquid Chromatography
Consumer concerns and federal regulations make pesticide residue analysis an important component of ensuring food safety. It is desirable to rapidly and reliably screen samples for a large number of pesticides in as few methods as possible. Comprehensive pesticide screening is typically performed using both LC-MS and GC-MS techniques on dedicated MS platforms. For this analysis, both UPLC-MS/MS and APGC-MS/MS pesticide residue analysis was performed on a single mass spectrometer, with a changeover time from LC-MS/MS to GC-MS/MS of less than 30 minutes. The same sample extracts prepared from various fruit and vegetable commodities were run on both chromatography systems that were coupled to the same tandem quadrupole mass spectrometer. Each method targeted a list of approximately 200 compounds each, monitoring for at least two MRM transitions for each compound. Standards for data quality were taken from the SANTE Guidelines (11945/2015). In the four matrices analyzed, >96% of compounds were detected at 10 µg/kg, with a majority of compounds detectable below 1 µg/kg on both UPLC and APGC. The coefficient of determination on matrix extracted calibration curves were generally > 0.995 and ion ratios fell within 30% of the reference value. The RSD was < 10% for upwards of 90% of the compounds detected at the 10 µg/kg level. The data presented demonstrates the ability to increase compound coverage on a single mass spectrometer with the flexibility and reliability of performing UPLC-MS/MS and APGC-MS/MS for routine multiresidue pesticide analysis.

**Keywords:** Food Contaminants, GC-MS, Liquid Chromatography/Mass Spectroscopy, Pesticides

**Application Code:** Food Safety

**Methodology Code:** Liquid Chromatography/Mass Spectrometry
The United States Food and Drug Administration (FDA) requires disclosure of trans fat content of conventional foods and dietary supplements. Sample preparation for the most common methods involves derivatization of the hydrolyzed free fatty acids, converting them over as methyl esters (FAMES). Overall fatty acid composition can be determined with Polyethylene Glycol (PEG) capillary columns where double bonds of unsaturated fatty acids are mainly of cis configuration. Separation and differentiation of cis / trans structures require highly polar columns containing biscyanopropyl stationary phases. This work examines FAMES using a high-cyano containing capillary column by AOCS Method Ce-1j-07, AOAC Method 996.06, as well as other applications.
The challenge in developing the analytical assessment of unexpected excess contaminations in infant formula has been the most significant project to address the widespread issue of food safety and security. Foodomics based on metabolomics techniques provides powerful tools for the detection of tampering cases with intentional contaminations. However, the safety and risk assessments of infant formula to reveal not only the targeted presences of toxic chemicals, but also molecular changes involving unexpected contaminations, have not been reported. In this study, a huge amount of raw molecularly-based signals from infant formula was analyzed using the reversed phase (RPLC) and hydrophilic interaction (HILIC) chromatography with time-of-flight (TOF)/MS processed by a principal component analysis (PCA). Based on these results, the PCA plots visualized signature trends in the complex signal-data batches from each excess contamination of detectable exogenous chemicals by LC-MS. These trends in the different batches from a portion of excess chemical contaminations such as pesticides, melamine and heavy-metals and out-of-date product can be visualized from spectrally-discriminated infant formula samples. The PCA plot provides possible attempts to maximize the covariance between the stable lot-to-lot uniformity and excess exogenous contaminations and/or degradation to discriminate against the molecularly-based signals from LC-MS detection (Figure).

Abstract Text

Keywords: Analysis, Food Identification, Food Science, HPLC
Application Code: Food Safety
Methodology Code: Liquid Chromatography/Mass Spectrometry
Food Safety - LC, GC, and MS

Toxicity, Accumulation, and Trophic Transfer of Metals and Metalloid in Food Chain Species from a Pond Fed Aquaculture Waste

Freshwater systems provide essential ecological services to humans but aquatic species are threatened by many anthropogenic processes. Predatory fishes such as Largemouth Bass Micropterus salmoides in pond settings can have a range of prey options. Therefore, their nutritive value to Largemouth Bass may differ markedly on how it supports the growth of other predators. We collected by seine 200 g live weight each of the most abundant prey species in a catchment pond fed by drainage of aquaculture research facility. The species included Bluegill Lepomis macrochirus, Black Crappie Pomoxis nigromaculatus, smaller Largemouth Bass, Brook Silversides, Northern Crayfish Orconectes virilis, and Burrowing Mayflies (Hexagenia sp.). Samples were frozen, lyophilized, and pulverized prior to analysis. Proximate composition, amino acid profile, and other parameters were determined using standard methods. Over 19 (Ca, Co, Cr, Cu, Fe, K, Li, Mg, Mn, Mo, Na, Ni, S, P, Sn, V, Zn, As, Pb, Cd, and Hg) elements were determined by inductively coupled plasma - mass spectrometry. Hg was determined by atomic absorption spectrometry. Method accuracy was evaluated using Dogfish liver for trace metals and other constituents (DOLT-5), Dogfish muscle certified reference material for trace metals (DORM-2), and Fish protein certified reference materials for trace metals (DORM-4). The results of the method validation, nutritional value, human exposure to trace metal through ingestion of the fishes will be presented. Additionally, the safety of consumption will be evaluated based on comparative studies to threshold regulatory values.


Keywords: Environmental Analysis, Food Contaminants, Food Safety, ICP
Application Code: Food Safety
Methodology Code: Atomic Spectroscopy/Elemental Analysis
The aim of this study was to monitor levels of cadmium (Cd), arsenic (As) and lead (Pb) grown in western Canada in peas and lentils over a three year period from 2014 to 2016 to obtain information on their occurrence and variation amongst growing years. The Grain Research Laboratory, a division of the Canadian Grain Commission (CGC), has programs in place to monitor the levels of specific elements in Canadian grains for food safety issues including Cd, As and Pb.

The samples were collected by the CGC Harvest Sample program. This is a mutually beneficial program where producers voluntarily send in their new crop sample for unofficial grading and quality information. Composites were prepared from submitted samples by crop region and factors like class, grade, variety and seed size dependent on the type of pulse. Samples were prepared by microwave digestion and analyzed by ICP-MS. The number of samples analyzed per crop year ranged from 35 to 52 for peas and 33 to 43 for lentils.

All results for As and Pb were less than 0.05 mg/kg, the method limit of quantitation. The overall Cd means (in mg/kg) for all samples analyzed for the three crop years were peas 0.020 and lentils <0.01 (the method limit of quantitation). There was no difference in Cd concentrations amongst the three years studied. All results for peas and lentils were less than the maximum limit (in mg/kg) established by the Codex Alimentarius Commission for pulses 0.1 for Cd and 0.2 for Pb.

Keywords: Food Contaminants, Food Safety, ICP-MS, Trace Analysis
Application Code: Food Safety
Methodology Code: Atomic Spectroscopy/Elemental Analysis
Simultaneous Determination of 19 Elements in Beverages by ICP-MS

19 elements in beverage were detected simultaneously by SHIMADZU ICPMS-2030 inductively coupled plasma mass spectrometry including the high concentrations of elements such as the sodium, calcium and the trace elements such as arsenic, lead. The experimental results show that the working curve of each element is linear with wide range. The correlation coefficient is up to 0.9999, and the detection limit of the method is 0.0005~0.05 g/L. The method was validated by adding standard recovery experiment and dilution experiment, and the recoveries were 92%~108% and 91~108% respectively. The method is simple, fast, accurate and reliable, which can meet the content analysis of various elements in beverage.

Keywords: Beverage, ICP-MS
Application Code: Food Safety
Methodology Code: Atomic Spectroscopy/Elemental Analysis
Total Elemental Analysis of Food Samples with ICP-OES and ICP-MS

The measurement of toxic, essential and nutritional elements in food has, through regulatory drivers and health-conscious consumers, become a routine part of food quality monitoring. Alongside regulatory compliance, it is necessary to monitor potentially toxic contaminants that could enter the food chain via a series of pathways including, but not limited to, industrial pollution or environmental contamination. For these reasons, it is essential to have a simple, robust, multi-elemental analysis method for major and minor concentrations of elements in food.

ICP-OES and ICP-MS are sensitive and rapid techniques with wide linear dynamic range and as such are ideal tools for the analysis of trace and major analytes in food in one analytical run. The accuracy of these techniques is demonstrated through the analysis of food based certified materials following microwave digestion. The principal challenge for trace elemental ICP-based techniques are interferences that stem from the complex food matrix, the reagents used to prepare the sample and the plasma source. This paper reviews different strategies including collision/reaction cell (CRC) strategies in triple quadrupole ICP-MS for the accurate analysis of trace elements in more challenging matrices.

Keywords: Food Safety, ICP-MS, Plasma Emission (ICP/MIP/DCP/etc.), Spectroscopy
Application Code: Food Safety
Methodology Code: Atomic Spectroscopy/Elemental Analysis
The analysis of pesticide residues in food is challenging because of the high number (typically >800) of substances that need to be analyzed in a diverse range of complex matrices. In order to achieve this within a routine environment, sensitive and selective LC and GC triple quadrupole MS systems are used. However, when such large numbers of compounds need to be analyzed it is an advantage to employ a system with full scan acquisition provided the performance is similar to that of triple quadrupole techniques. A generic acquisition based on full scan MS is more straightforward and provides additional information compared with multiple reaction monitoring by triple quadrupole mass spectrometry. It also increases the scope of the analysis, as target compounds are selected post acquisition. In order to obtain sufficient selectivity in the full scan mode, high resolution/high mass accuracy MS instruments are required.

In this study, the quantitative performance of the Thermo Scientific™ Exactive™ GC Orbitrap™ GC-MS system was evaluated for the routine analysis of GC-amenable pesticides in leek, orange and tomato matrices. In total 99.3% of the 153 pesticide/matrix combinations were detected below the respective maximum residue limits (MRLs) with linearity in compliance with the SANTE/11945/2015 criteria. Linearity was assessed using matrix matched standards across a concentration of 0.5-500 µg/Kg. In all cases, the coefficient of determination (R2) was >0.99 for each pesticide from its LOD to 500 µg/Kg in the three matrices. Acquisition using 60,000 FWHM resolving power (at m/z 200) reduces matrix interferences and increases confidence in results when screening for pesticides in complex sample matrices. Sub ppm mass accuracy was achieved for all compounds over a wide concentration range ensuring that compounds are detected with confidence at low and high concentration levels.

Keywords: Food Contaminants, Food Safety, Gas Chromatography, Gas Chromatography/Mass Spectrometry
Application Code: Food Safety
Methodology Code: Gas Chromatography/Mass Spectrometry
Food Safety - LC, GC, and MS

Fully Automated Determination of 3-MCPD, 2-MCPD, and Glycidol in Edible Oils by GC/MS Based on Method AOCS Cd 29c-13

3-MCPD, 2-MCPD, and Glycidol and especially their fatty acid esters are process contaminants that are formed, for example, when edible oils and fats are refined. At least some of the above-mentioned substances are classified as potential human carcinogens, a fact which has prompted the introduction of rules and regulations that specify tolerable daily intake values and maximum levels in edible oils.

This paper describes a solution for fully automated determination of 3-MCPD, 2-MCPD, and Glycidol in edible oils based on the reliable indirect method AOCS Cd 29c-13, similar to the ISO 18363-1 and DGF C-VI 18 (10) methods that are essentially identical. The edible oil sample is divided into two parts (assays A and B). Both are saponified using a Sodiumhydroxymethanol solution, but different quenching methods are used. In assay A, free Glycidol is converted to 3-MCPD using acidic quenching conditions in the presence of chloride. In contrast, for assay B, the quenching reagent is an acidic chloride free salt solution, in which free Glycidol is not converted into 3-MCPD. Following derivatization, the 3-MCPD amounts in both samples are determined by GC/MS as Phenylboronic acid (PBA) esters. Assay B is used to determine the amount of 3-MCPD in the sample while assay A provides the combined amounts of 3-MCPD and Glycidol. The amount of Glycidol is determined as the difference between the assay A and assay B results. The work presented here involves an automated evaporation step as prescribed in the abovementioned official methods. This ensures that for most matrices, the required limits of detection can be reached using a single quadropole mass spectrometer (MSD). A further important aspect of the evaporation step is that it removes excess derivatization reagent, which could otherwise build up in the GC/MS system and influence system stability.

Keywords: Automation, Food Safety, GC-MS, Sample Preparation

Application Code: Food Safety

Methodology Code: Gas Chromatography/Mass Spectrometry
Aromatherapy products are essential oils that are chemical mixtures extracted from plant material by boiling, steam distillation, or expression methodologies. The consumer understands that essential oil is a wholesome, natural substance and not a synthetic, industrially-made product like prescription drugs. Consumers also trust that essential oils are not chemically modified after extraction.

Despite product labels proclaiming “Pure” and “Natural”, sources within the aromatherapy industry identify product abuses, chemical alterations and inappropriate species substitutions. Chiral GCMS with beta-cyclodextrin capillary columns of different selectivity were used to determine the chiral compound composition of a selection of 42 locally and internationally available aromatherapy products. Fifteen (36%) contained synthetic enantiomeric constituents. Racemic mixtures were scored as “Synthetic”.

Bioactive enantiomers are right or left handed molecules that like human hands that cannot be superimposed on each other. Within the same species nature usually produces only the single enantiomer that exerts the desired biological benefit. Contrarily, when the same chemical is synthetically manufactured, the result is a racemic mixture of both enantiomers in predictable proportions. The un-natural enantiomer can cause irritation or an adverse immune response.

A review of purity testing practices of each manufacturer and a review of national and international regulations is discussed.

References:

4. Biological Activities of Alpha-Pinene and Beta-Pinene Enantiomers, Molecules 2012,17, 6305-6316.

Keywords: Chiral Separations, Food Safety, Forensic Chemistry, Natural Products
Application Code: Food Safety
Methodology Code: Gas Chromatography/Mass Spectrometry
In recent years there has been a dramatic expansion in the number of pesticides utilized in food products, especially in emerging markets and commodities. With this expansion, analytical techniques with the ability to acquire comprehensive non-targeted data have become increasingly important. However, large lists of target compounds can be challenging and time-consuming to maintain, often requiring multiple standard and sample injections in order to develop methods for different matrix interferences and analytical conditions. This presentation will showcase the creation and utilization of a target list of pesticides using software tools designed to make processing comprehensive data faster, easier, and more effective. With enhanced flexibility in data processing parameters, fewer injections are needed to fully develop an easy-to-update, automated data processing method for targeted analytes. A proposed target list containing over 200 commonly used pesticides serves as a starting point for the development of a new pesticide analysis solution kit. The ability to easily add emerging pesticides to the stock list will also be demonstrated. This workflow utilizes a robust benchtop time-of-flight GC/MS system capable of femtogram level sensitivity and is expected to compete favorably with current workflows employing systems requiring selected ion monitoring (SIM) or MS/MS to achieve desired sensitivities.
Pesticides are widely used in food production and to manage various pests, and as such there is a need to monitor the presence of residues in food. The detection, quantification, and confirmation of pesticide residue in food at trace levels requires sensitive, selective and robust analytical instrumentation. With ever increasing pressure to analyze contaminants at very low levels in greater number of samples and with shorter turnaround times, laboratories seek continuous improvements in analytical instrumentation to increase productivity and minimize downtime.

This study describes a completely optimized workflow for multi-residue pesticides analysis in baby food using superior sensitivity on a Thermo Scientific GC-MS/MS system. Accurate and sensitive detection, quantification, and identification of pesticides in baby foods is of particular importance as babies are more vulnerable to adverse health effects from these chemicals. In this work, various commercially available fruit and vegetables based baby-food samples were subjected to an optimized QuEChERS extraction method to isolate the pesticide residue using acetonitrile as the final extraction solvent. Direct analysis of extracts in acetonitrile is desired to avoid the need for solvent exchange to a more GC amenable solvent. The QuEChERS extracts were then analyzed for pesticide residue content using a fast targeted timed-SRM method for >200 pesticides including priority pesticides. The performance of the method was assessed and various analytical parameters investigated. The data presented in this study demonstrates unprecedented method performance from sample preparation to sensitive and robust GC-MS/MS analysis in addition to automated data processing and reporting capabilities.

Keywords: Gas Chromatography/Mass Spectrometry, GC-MS, Pesticides, Tandem Mass Spec
Application Code: Food Safety
Methodology Code: Gas Chromatography/Mass Spectrometry
Food Safety - LC, GC, and MS

Determination of 217 Pesticide Residues in Vegetables and Fruits by GC-MS/MS

Introduction:
As serious food safety problems are constantly being exposed, food safety has become the focus of attention. Nowadays the acute pesticide poisoning has become a common concerned social problem of the world. Eating vegetables and fruits with excessive amount of high toxic organophosphorus pesticide and carbamate pesticides can easily cause poisoning and even death. As the old methods are tedious and time-consuming, it is urgent to develop a rapid, cheap and accurate detection method of pesticide residues. In this paper, GC-MS/MS combined with effective pretreatment (SHIMADZU-GL WondaPak QuEChERS) and Smart Database Pesticides enables labs excluding interferences from other compounds-allowing you to achieve accurate qualitative and quantitative analysis of 217 pesticide residues in vegetables and fruits.

Method:
Instrument: GCMS-TQ8050 (Shimadzu Corporation, Japan)
Sample preparation:
1. weight 10g sample, put into a 50 mL centrifuge tube, add 10 mL acetonitrile.
2. Adding QuEChERS salt extraction package, shock for 1min , centrifuge for 5 minutes at 4200r/m
3. Transfer 6 mL supernatant into a d-SPE purification tube, vortex for 1min, centrifuge for 5 minutes at 4200r/m, absorb 4mL supernatant into 10mL glass centrifuge tube, blow to dry with nitrogen flow
4. dissolve with 2mL ethyl acetate, analyzed by GC-MS/MS

Results and conclusions:
Most compounds are in good linearity within 0.5~200ng/mL, with correlation coefficient more than 0.999. The limits of quantification (LOD) of 217 compounds are between 0.01~1.60ng/mL. The reproducibilities were good with the relative standard deviations (RSD) less than 9.35%. The average recoveries of all compounds are between 74.87~107.44% at spiked level of 20ng/mL. The results show that it’s a quick, convenient, reliable and robust analytical method for determining the amount of 217 pesticide residues in vegetables and fruits.

Keywords: Food Safety, Gas Chromatography/Mass Spectrometry, Pesticides, Quantitative
Application Code: Food Safety
Methodology Code: Gas Chromatography/Mass Spectrometry
The presence of antimicrobial residues in animal products, resulting from treatment of livestock with pharmacological drugs can pose a severe risk to consumer health such as generating unknown resistant pathogenic bacteria. To ensure food safety, food products of animal origin must be confirmed as the maximum residue limits (MRLs) are not exceeded by antimicrobial residues. Highly selective analytical methods are essential to assay these compounds in complex matrices. However, a determination of antimicrobials using a single detection method can be challenging because all antimicrobials do not have even a partly common chemical structure.

In this study, a simple HPLC equipped with fluorescence detector (RF) and photodiode array detector (PDA) was used for screening antimicrobials in meat (beef, pork and chicken muscle) with limits of detection below the MRLs, eg 10 μg/kg. Sample preparation was performed by selecting either the QuEChERS method or liquid–liquid extraction depending on the compound classes. A Shim-pack FC-ODS was used as an analytical column. The employed mobile phases for gradient elution were composed by mixing following two solutions, A) A (sodium) phosphate buffer containing sodium perchlorate, B) A mixture of acetonitrile–methanol. Among three main classes of antimicrobials, sulfonamides and antifolates were simultaneously detected using six different monitoring wavelengths of PDA. Quinolones were detected using two different combinations of excitation and emission wavelengths of RF and a single wavelength of PDA. Sample preparation and multi-wavelength detection were optimized for the screening of 24 antimicrobials then successfully applied to meat samples from different animal species. No contamination with the antimicrobials in any meat sample were observed. As a result, shorten screening time and improved reliability of determination were obtained through the method package compared to those of present methods such as the paper disc method.

Keywords: Food Safety, HPLC, Method Development
Application Code: Food Safety
Methodology Code: Liquid Chromatography
As part of the US Department of Treasury the Alcohol and Tobacco Tax and Trade Bureau (TTB) is responsible for regulating the use of ethanol in products manufactured in the United States and collecting revenue (excise tax) generated from such use. One component of TTB’s jurisdiction is the regulation of nonbeverage alcohol (NBA) products. These products include medicines and medicinal preparations; food products; flavors and flavoring extracts; and perfumes manufactured using taxpaid distilled spirits and which are unfit for beverage purposes. Manufacturers may claim drawback of the taxes paid on eligible spirits which were used in the production of approved NBA products. Determination of alcohol content by GC is a necessary step in evaluation of NBA products submitted for drawback approval, but many of these products (e.g. sauces, syrups, gels, cakes, ice creams) are not amenable to liquid injection. Instead, the headspace GC-MS method described herein was developed to quantify the alcohol content of NBA samples containing up to 18% ABV. All samples were homogenized and diluted with water prior to analysis. The linear range for the method was 0.05-2% ABV with a coefficient of determination (r² > 0.999). Intra- and inter-day repeatability and reproducibility were verified and the use of deuterated ethanol as an internal standard ensured that the method was both robust and relatively insensitive to matrix effects. The new headspace method has been demonstrated to be accurate, precise, and robust and can be used for the determination of alcohol content in NBA products.

Keywords: Food Safety, Gas Chromatography/Mass Spectrometry, Headspace, Method Development
Application Code: Food Safety
Methodology Code: Gas Chromatography/Mass Spectrometry
How to Obtain Elemental Formula with Your GC-MS

Sample identification with GC-MS is typically based on the use of library search such as NIST. However, many compounds such as novel synthetic organic compounds are not included in the library. Thus, there is growing need to obtain the sample elemental formula from unit resolution single quadrupole GC-MS data.

We developed the Tal-Aviv Molecule Identifier (TAMI) software that automatically confirms or rejects NIST library identification. Alternatively the TAMI software improves the quadrupole MS mass accuracy and inverts the combined improved mass accuracy and molecular ion isotopomeric pattern into elemental formulae.

Recently we developed a new algorithm for improved mass accuracy named QMass which provides typically 40 ppm mass accuracy and no worse than 100 ppm from both Agilent 5977 Chemstation/MassHunter data files and Thermo ISQ Chromeleon data files. We explored the efficiency of combined improved mass accuracy and isotope abundance analysis for the provision of elemental formulae and found it to be equivalent or better than of 1 ppm mass accuracy alone. Furthermore, TAMI is easy to use and can be operated with standard centroid data files.

Thus, TAMI upgrades unit resolution quadrupole based GC-MS to be similar in sample identification capability to costly accurate mass GC-TOF-MS. You are invited to send us your files for its demonstration.

Keywords: Elemental Analysis, Gas Chromatography/Mass Spectrometry, GC-MS, Software
Application Code: Other
Methodology Code: Gas Chromatography/Mass Spectrometry
The most significant shortcoming and Achilles Heel of GC-MS is its inability to analyze relatively non-volatile and thermally labile compounds. GC-MS with Cold EI is based on electron ionization of vibrationally cold molecules in supersonic molecular beams (SMB) (hence the name Cold EI) and on interfacing the GC and MS with SMB while replacing the EI ion source with Cold EI fly-through ion source.

In GC-MS with Cold EI the GC elution temperatures can be significantly lowered upon the reduction of the column length and increase of carrier gas flow rate and over 200ºC lower elution temperatures were demonstrated. Furthermore, via using high column flow rate, lower injector temperatures can be used and sample degradation at the Cold EI fly-through ion source is inherently eliminated. Thus, extended range of thermally labile, polar and low volatility compounds are amenable for analysis. Furthermore, the ionization of cold molecules results in enhancement of the molecular ions and GC-MS with Cold EI further facilitates much shorter analysis times.

Several examples of extended range of GC-MS with Cold EI applications will be demonstrated in the analysis of large hydrocarbons (all with molecular ions), full range of all the organic explosives, large polar drugs, thermally labile "LC-MS" pesticides, lipidomics analysis including free fatty acids, cholesterol diglycerides and triglycerides and service GC-MS for the analysis of synthetic organic compounds including the provision of their elemental formula for publications. Consequently, GC-MS with Cold EI bridges the gap with LC-MS.
There is a trend towards applications using fast GC/MS for routine analysis in a wide range of fields involving complex mixtures. This fast analysis time requires unique capabilities including: a GC with fast column heating and cooling for high throughput, a mass spectrometer with high data acquisition speed and a fast response ion source in order to correctly characterize narrow peaks. To make sense of the resulting compressed data, the software needs the capability of automatic peak finding and deconvolution of co-eluting peaks for correct and meaningful identifications and quantitation.

The performance of an instrument capable of combining Fast GC and TOFMS, where the total analysis time including time between runs is less than 3 minutes, will be demonstrated.

The Fast GC is manufactured by Aviv Analytical. It is easily mounted to a standard GC detector port. The GC column used is shorter than typical (~3m). It is installed in a standard way in the inlet and fed into the low thermal mass heating tube of the Fast GC. The column passes again through the GC and into the transferline between the GC and TOFMS. The metal tube in the Fast GC is resistively heated by electrical current to obtain fast heating rates (typically 200-600°C/min). The GC has a pressure program and the oven is held at a constant temperature similar to the transferline. The Fast GC parameters are controlled with separate electronics box and standalone software.

The TOFMS is LECO Corporation’s Pegasus® BT. Since the peak widths in rapid separations are narrower than typical chromatographic peaks(100-200ms), a high acquisition speed(up to 80 s/s) is used to get the necessary number of mass spectral points across the peak for appropriate deconvolution and quantitation. With such a large number of peaks eluting in a short time there are multiple co-elutions. Examples where automatic peak find and deconvolution algorithm allow peak identification within these co-elutions will be demonstrated.
GC/MS library search is a mainstay application as part of the identification, confirmation and quantitation of known and unknown compounds. In spite of its huge popularity, library search results are sometimes ambiguous and in some instances, can fail to produce practically useful results. This is due to several reasons including unresolved or co-eluting chromatographic peaks (mixtures), column bleed and background interferences, or simply the absence of the target compound in the library. While there are manual methods to work around some of these limitations, they tend to require a high degree of user expertise and can be tedious and time consuming.

Accurate mass lineshape calibration has demonstrated that with conventional single quad GC/MS, accurate mass measurement (to 0.001 Da) combined with spectral accuracy analysis can enable these instruments to perform formula identification in the absence of spectral interferences in relevant m/z ranges (1). This provides additional information to validate conventional GC/MS library search but is typically a manual process and does not address the problem of unresolved chromatographic peaks or background/spectral interferences.

In this presentation, we will present a new method to automatically detect and deconvolve unresolved chromatographic peaks. The method utilizes information from experimentally measured spectra present in the GC/MS libraries and leverages the corrected spectral lineshapes (spectral accuracy) to provide orthogonal information to identify compounds not present in the library or enhance confirmation for those already in the library. The entire process can be automated using a flexible scripting language that can be adapted to accommodate regulatory requirements or to address the specific needs of applications and/or laboratories.

Keywords: Chemometrics, GC-MS
Application Code: General Interest
Methodology Code: Gas Chromatography/Mass Spectrometry
We conducted physical property analyses of select little cigar products to study the variation both within and between brands and related these observations to chemical differences observed in their mainstream smoke deliveries. In this study, we analyzed 60 convenience little cigar brands purchased in the metropolitan Atlanta area for common physical properties including filter ventilation, pressure drop, filter and rod length, diameter, and tobacco weight. Physical properties relate how engineering differences of products impact delivery and levels of smoke-born chemicals such as hydrogen cyanide (HCN) and volatile organic compounds (VOCs). Within brand variations are important to assess as these could contribute to mainstream smoke measurement uncertainties. Little cigar products were conditioned according to ISO 3402 standards. Physical properties measurements were taken for n=7 replicates of 60 little cigar brands using a Cerulean C2 Testing Unit. For HCN and VOCs analysis, replicates were smoked under ISO and Canadian Intense (CI) regimes on a linear Borgwaldt machine and samples were analyzed using Agilent gas chromatography/mass spectrometry (GC/MS) instruments. These results were compared with previously reported physical properties of cigarettes.

Physical property variations were observed among brands, mainly due to differences of engineering designs, as well as within brands. For example, filter length differences for one particular brand were up to 82% resulting in a 46% difference on HCN deliveries under ISO conditions. We also observed among all brands an inverse correlation between ventilation and HCN levels under the ISO regime. We conclude that physical property analysis is a useful tool for studying products across and within product categories and potentially linking product design to the machine smoked deliveries.

Keywords: Analysis, Consumer Products, Gas Chromatography/Mass Spectrometry
Application Code: High-Throughput Chemical Analysis
Methodology Code: Physical Measurements
Comprehensive two-dimensional gas chromatography (GCxGC) combined with time-of-flight mass spectrometry (TOFMS) is a well-established method for characterization of petroleum samples because of the ability to separate structural classes of hydrocarbons into distinct bands of analytes that can then be identified with library-matched mass spectra. However, transferring traditional one-dimensional gas chromatography methods to optimized two-dimensional methods can be daunting because of the sheer number of options available for setting up commercial GCxGC systems: orthogonal column phases, secondary oven temperature offsets, modulator temperatures, and second dimension separation lengths. Using a new online tool, SimplyGCxGC, the process of translating an existing analysis into an optimized GCxGC method is experimentally determined for a sample of diesel fuel. Calculations that maximize use of two-dimensional space and minimize analyte wrap-around are applied to user-controlled parameters to make the most of peak-capacity increases and further enhance the chromatographic resolution gains from the additional dimension of separation.

Keywords: Chromatography, Fuels\Energy\Petrochemical, Gas Chromatography/Mass Spectrometry, Method D
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Gas Chromatography/Mass Spectrometry
We have been examining and testing a range of building and construction materials for their suitability and level of risk to the Library of Congress’ collections. The majority of tested materials have been found to fail the Oddy metal coupon test, often in a rather unusual or spectacular manner. The Oddy Test is a historic, but subjective, assessment of the impact of storage materials on metal coupons as a predictive test for hazard to collections. We have been examining the compounds emitted by these materials and how they interact with the copper, silver and lead coupons used in the Oddy test. Through the use of thermal desorption gas chromatography mass spectrometry, we are able to identify and quantify the compounds emitted from a material. In addition, other analytical tools are being utilized to examine what compounds are depositing or reacting at the surface of the metal coupons during exposure to elevated temperatures and humidity. Coordinating and comparing these chemical analyses with the results from the Oddy test are improving our ability to understand the mechanism(s) behind the failure of the Oddy test and, in turn, guide future product selection. While the goal of this screening is to select materials that produce a minimal risk to the collection, this risk often cannot be entirely removed by product choice alone (i.e. all tested materials damage a metal coupon to some extent). As a means of mitigating the residual risk from volatiles, we are also examining and characterizing commercially available sorbent materials for their selectivity, capacity, functionality and adsorption/desorption characteristics.

Keywords: Art/Archaeology, GC-MS, Quantitative, Volatile Organic Compounds
Application Code: Art/Archaeology
Methodology Code: Gas Chromatography/Mass Spectrometry
**Abstract Title:** New Ionic Liquid Stationary Phase Selectivity Evaluations

Ionic liquid stationary phases have been demonstrated to provide very unique selectivities compared to traditional polysiloxane or polyethylene glycol based phases. This selectivity is made possible due to the various combinations of cations and anions that are available along with spacer groups used to prepare these germinal dicaticionic phases. Columns prepared with di- or tricationic phases and newer phases that contain a PEG or branched chain alkyl linkage chain have the ability to perform many of the same applications as columns made with polysiloxane polymer or polyethylene glycol stationary phases of similar polarity, but with slight elution order changes. Many times this results in increased resolution and/or shorter run times. This paper will compare and contrast the selectivity of the ionic liquids stationary phases with traditional phases of similar or like selectivity’s for applications with a variety of different sample types from a number of industries including water analysis, petrochemical, pharmaceutical, environmental, food and beverage and flavor and fragrance.

**Keywords:** Capillary GC, Flavor/Essential Oil, GC Columns

**Application Code:** General Interest

**Methodology Code:** Gas Chromatography/Mass Spectrometry
New Sampling Approaches Offering Compatibility with Thermal Desorption for Enhancing Sensitivity and Sample Characterization in GCMS Analysis

Thermal desorption (TD) combined with GC(MS) has long been used as a valuable tool in the sampling and pre-concentration of (S)VOCs in vapour phase samples, particularly for environmental and workplace air monitoring. New developments in sampling technologies for TD, including high-capacity sorptive extraction, has extended the applicability of TD to liquid and solid samples, with the capacity for both immersive and headspace sampling to extract components from within the sample prior to pre-concentration and analysis.

Thermal desorption offers a number of well-known advantages over solvent-extraction methods for a wide range of VOCs and SVOCs, including greatly improved sensitivity due to the avoidance of dilution, high extraction efficiency, and efficient transfer/injection into the GC. Furthermore, high-capacity sorptive extraction offers an extension to SPE & SPME methods which is simple to employ and, when combined with TD, offers a versatile method for gaining complementary information to that contained using other sampling approaches.

This multi-facted sampling approach has been applied to a number of different sample types, including environmental matrices, foods, beverages and clinical samples, examples of which will be presented. The benefits of using TD sample introduction extend to the ability to re-collect sample for repeat analysis, assisting with method validation and eliminating the need to perform repeat extractions on limited sample quantities.

Keywords: Automation, Sample Introduction, Sample Preparation, Thermal Desorption
Application Code: General Interest
Methodology Code: Gas Chromatography/Mass Spectrometry
Abstract Text

Dicationic ionic liquid (IL) gas chromatography (GC) stationary phases are getting popular as they show higher thermal stabilities, variety of polarities, and unique selectivities. Generally, the traditional stationary phases show one dominant type of chemical interaction, while IL phases show multiple solvation interactions and this is the origin of their unique selectivity towards a variety of molecules with different functional groups. The selectivities and polarities of dicationic ILs can also be altered by variations in their structural moieties i.e. cations, linkage chains, and anions. Among ILs, the phosphonium based ILs show different selectivities compared to the nitrogen based ILs (imidazolium, pyrrolidinium etc.).

But, not many phosphonium IL GC stationary phases are designed except for alkyl and phenyl substituents on the phosphorous. As the selectivities can be modified by variation of substituents, a series of new phosphonium dicationic ILs were designed by using different substituents (phenyl, pyridyl, furyl etc.) and evaluated as GC stationary phases. The phosphonium IL phases were applied in the analysis of 2,3,7,8-tetrachlorobenzo-p-dioxin (TCDD) enantiomers, fatty acid methyl esters (FAMEs), and polynuclear aromatic hydrocarbons (PAHs). The Phosphonium ILs showed interesting selectivities towards these compounds and higher thermal stabilities.

Keywords: Chromatography, Gas Chromatography, GC Columns, PAH
Application Code: General Interest
Methodology Code: Gas Chromatography
Polycyclic aromatic hydrocarbons (PAHs) is a large class of environmental pollutants studied worldwide due to their carcinogenic and mutagenic properties. The analytical methods for determination and quantitation of PAHs consist of gas chromatography-mass spectrometry and reverse-phase liquid chromatography (RPLC) coupled with ultraviolet/visible (UV/vis) and/or fluorescence detection (FLD). This study provides a new analytical approach for the identification and quantitation of molecular mass (MM) 302 Da PAH isomers in standard reference material (SRM) 1597a using RPLC coupled with real time synchronous FLD. Normal-phase liquid chromatography fractionation using an aminopropyl (NH2) semi-prep column was utilized prior to RPLC analysis for sample cleanup purposes. The MM 302 Da PAH isomers were determined in SRM 1597a fractions based on RPLC retention times coupled with unique synchronous fluorescence spectral profiles. Quantitative values for selected PAHs in SRM 1597a were obtained by the new analytical method and compared to the assigned mass fraction values provided by the National Institute of Standards and Technology.
Since their initial development in 2003, preparation of electrostatically attached hyperbranched anion exchange materials has become widely used in ion chromatography. In my initial work, I utilized methylamine and 1, 4-butanediol diglycidyl ether to prepare a wide variety of different anion exchange materials with unusual features: ion exchange capacity doubles with each reaction cycle and selectivity progressively shifts as the number of reaction cycles increases. This chemistry platform is highly versatile but thus far has largely been limited to use of the reagents above. However, this synthetic platform can make use of a wide variety of amines and epoxy containing moieties suitable for producing a wide variety of anion exchange materials. In this work I will describe a novel reagent system that provides unprecedented selectivity, illustrating the versatility of this synthetic platform for the preparation of ion-exchange phases suitable for use in ion chromatography and HPLC. This new class of anion exchange materials exhibiting exceptionally low retention of multivalent species even when using low ionic strength eluents while at the same time providing good retention of monovalent species. The synthesis and properties of this new class of anion exchange materials will be described, and applications of such materials will be demonstrated.
Integration of solid phase extraction or column cleanup with chromatographic analysis may take two approaches: 1) Direct coupling, in which a sample loaded in a cleanup column is completely transferred to the analytical column using a switching valve; 2) Indirect coupling, in which samples are treated like conventional offline cleanup. A portion of the collected fraction is then injected into the analytical column using a built-in auto sampler or by triggering the auto sampler from the HPLC. The direct approach can give a much higher sensitivity than indirect approach, whereas the indirect approach is easier to find suitable cleanup columns and faster for method development. One of the major challenges in a direct approach is finding suitable cleanup columns that can give effective cleanup and allow use of compatible mobile phase. If one cleanup column can not process at least 50 samples, an expensive device for loading cleanup columns need to be used which makes this type of online SPE device much more expensive than those based on indirect approach. This presentation will show how to avoid peak dispersion and prolong life span of a cleanup column in direct online SPE using analysis of PAHs in water as an example. We will also show how to increase sensitivity in indirect online SPE. Finally we will demonstrate how to combine these two online approaches into one device so that the disadvantages can be avoided.

Keywords: Automation, Liquid Chromatography, On-line, Solid Phase Extraction
Application Code: Environmental
Methodology Code: Liquid Chromatography
A rapid, sensitive, fast and inexpensive method has been developed for analysis of Quizalofop-p-Tefuryl. The separation of chiral compounds has been of great interest because pesticide activity depends on chiral isomer. Quizalofop-p-tefuryl chiral isomers were separated on chiral column Chiral Pak AS-H (250 mm. 4.6 mm x 5 m with 96:4 mix.) with n-hexane: IPA as mobile phase at flow rate 0.5 ml/min. The effluent was monitored by UV detection at 230 nm. Impurity profile of Quizalofop-p-Tefuryl was done with reverse phase HPLC and it was validated with different parameters namely specificity, LOD, LOQ, Precision and Accuracy. The current study describes a new HPLC method using simple mobile phase for the determination of Quizalofop-p-Tefuryl and its impurities (8 impurities). The method was validated and found to be accurate and precise. It was proved to be convenient and effective method for determination of Quizalofop-p-Tefuryl. Moreover, the lower solvent consumption along with short analytical run time leads to cost effective chromatographic method.

Keywords: HPLC
Application Code: Quality/QA/QC
Methodology Code: Liquid Chromatography
Ion chromatography with suppressed conductivity detection has been used routinely for ion analysis. To achieve lower detection limits, large volume of sample needs to be pre-concentrated onto a concentrator column before analysis. The presence of matrix ions will shift the retention time and distort the peak shape, leading to poor quantitation. To address this issue, a two-dimensional matrix elimination ion chromatography has been developed. The matrix ions were eliminated in the first dimension, while the ions of interest would be analyzed in the second dimension. In this presentation, we will show different strategies for implementing this technology to achieve low detection limit of ions in the presence of large amount of matrix ions. Its application for the drinking water analysis will also be shown.
Ion chromatography (IC) is a widely used analytical technique for determination of anionic and cationic analytes in various sample matrices. In modern IC systems, high purity acid, base, or salt eluents are generated electrolytically using deionized water as the carrier. The IC systems with electrolytic eluent generation make it possible to perform a wide range of ion chromatographic separations using only deionized water as the carrier. For many applications, these IC systems provide improved performance with increased sensitivity and the flexibility to perform isocratic and gradient separations. In addition to saving time, labor, and operating costs, these IC systems eliminate errors and problems associated with manual eluent preparation and offer users the benefits of simplicity and ease of use, and improved method reproducibility. In this presentation, we will report the recent developments of ion chromatography systems with electrolytic devices for generating high purity acid, base, and salt eluents using deionized water as the carrier stream. We will describe the principles and operation of the new electrolytic eluent generation platforms, and demonstrate the advantages of using these devices to achieve fast and high resolution ion chromatographic separations of various target analytes including inorganic anions and cations, organic acids, and carbohydrates under isocratic and gradient separation conditions.

Keywords: Environmental/Biological Samples, Food Identification, Instrumentation, Ion Chromatography
Application Code: General Interest
Methodology Code: Liquid Chromatography
Efforts to miniaturize liquid chromatographic (LC) systems are driven by the desire to reduce mobile phase consumption and by a need for field portable instruments capable of onsite analysis. In the latter case it is essential that all components of the instrument, including the detector, be reduced in size, and that they operate with low power consumption. UV absorption detectors incorporating light emitting diodes (LEDs) as the primary light source are capable of meeting the miniaturization requirements of field portable systems, and they provide detection limits in the nanomolar range for strongly absorbing compounds[1]. However, compared to benchtop detectors, including array detectors and mass spectrometers, the miniaturized systems are less sensitive and less selective. We have developed a dual mode detector that combines absorption and fluorescence detection in a single compact package. The addition of a fluorescence channel adds both sensitivity and selectivity to the detector, while the absorption channel maintains the response of the detector to a broad range of compounds.

The absorption channel of the dual detector is optically identical to the detector described in reference 1 with the exception of the insertion of a dichroic beam splitter between the two ball lenses that focus light from the LED onto the separation capillary. The beam splitter diverts fluorescence from analytes in the capillary, through an emission filter, and into a compact photon counting module. This epi-illumination scheme preserves the critical figures of merit for the absorption detector while adding significant sensitivity for fluorescent compounds and providing important additional specific information about the analyte in the form of a fluorescence-to-absorbance ratio.


Keywords: Capillary LC, Detection, Fluorescence, UV-VIS Absorbance/Luminescence
Application Code: General Interest
Methodology Code: Liquid Chromatography
Temperature assisted solute focusing (TASF) relies on the temperature dependence of solute retention to improve sample pre-concentration and analysis sensitivity. This is done by transiently cooling the head of the column, injecting a sample, then heating the head of the column to release the focused solute band. This is effective for capillary and 1 mm columns. TASF is most effective for solutes with large retention enthalpies. Ionizable analytes are typically separated at a pH where they exist as a neutral species, however, when mobile phase pH is close to the pKa of the analyte, buffer ionization enthalpy significantly contributes to the total adsorption enthalpy. The total adsorption enthalpy consists of analyte adsorption (charged and neutral species), analyte ionization, and buffer ionization enthalpies. In this system, a change in temperature leads to changes in analyte and buffer pKa, mobile phase pH, and retention factor of neutral and charged species. By choosing buffers with different enthalpies of ionization, the total adsorption enthalpy and response of chromatographic retention to temperature can be optimized for TASF. Our aim is to predict observed total adsorption enthalpy and retention factor to individual thermodynamic parameters for a selected buffer, analyte, temperature, and mobile phase pH value. Retention factors and enthalpy of adsorption for the charged and neutral species were measured on HPLC. Ionization enthalpies were measured using isothermal titration calorimetry. Dissociation constants were measured using potentiometric titration methods in mobile phase (75:25, Water:Acetonitrile). A program was written to predict observed total adsorption enthalpy by HPLC using these separately measured parameters. Predicted and experimental total adsorption enthalpies and retention factors follow the same trends with respect to chosen buffer, analyte, temperature, and mobile phase pH.

Keywords: Adsorption, Liquid Chromatography, Potentiometry, Titration
Application Code: Pharmaceutical
Methodology Code: Liquid Chromatography
The act of suppression results in removal of the counter-ion to the eluent while enhancing the signal for fully dissociated species. While this simplistic description works well when the sample concentration is low and well below the eluent concentration, with high levels of matrix ions there is a need to optimize the suppressor conditions to match the sample counter-ion concentration. If the matrix concentration exceeds the eluent concentration then the static capacity is consumed and may not be regenerated to facilitate good suppression. The net effect of the high level of matrix ions is the suppressor gets converted to the undesirable matrix ion form and can impact the signal intensities. In this presentation we explore quantitation under high matrix concentration conditions and study the impact on quantitation by reviewing response versus concentration curves for a variety of samples containing high levels of matrix ions. We present here the optimal suppressor design for achieving excellent quantitation for both electrolytically and chemically regenerated suppressors. Quantitation with four orders of magnitude is feasible with the present approach.
Hydrophilic interaction liquid chromatography (HILIC) has become a staple in chromatographic separations due to its ability to separate highly polar compounds. Retention in HILIC is mixed mode in nature, arising from partitioning into a water layer that forms on the surface of the polar stationary phase, and adsorption and/or other secondary interactions with the surface of the stationary phase.

While some notice has been given to cationic ion pairing agents, there is no direct mention of anionic ion pairing agents in HILIC. Ion pairing agents can have a dramatic effect on the retention and selectivity of a separation. Ion pairing agents have been well studied in reversed phase. This presentation will discuss the effect of anionic ion pairing agents on HILIC behavior for neutral, cationic, and anionic analytes over a range of percentage of acetonitrile and on a variety of classes of HILIC columns. Applications using both absorbance and mass spectrometric detection will be discussed.
A new alumino-silicate based spherical particles with average particle size of 10[micro]m were synthesized. The particles show narrow particle size distribution with D90/D10 of 1.6. BET specific surface area of the particles 250 m\(^2\)/g was observed. The alumino-silicate particles based stationary phase exhibited excellent HILIC and Normal phase selectivity. The new stationary phase chromatographic performances were compared to commercial silica based stationary phases. New alumino-silicate stationary phase showed excellent stability over wide pH range including high pH (pH 10) in HILIC mode. Thus, it may be one of the few HILIC phases where retention time changes and separation degradation over time does not observe. Selectivities for wide variety of pharmaceutically important compounds were examined and found to be quite different from silica.

Keywords: HPLC, HPLC Columns, Pharmaceutical, Separation Sciences
Application Code: Pharmaceutical
Methodology Code: Liquid Chromatography
Analytical methods for nitrite/nitrate determination in foods have often developed through application of technical approaches derived from the analysis of water. Many analytical methods have been recommended by both technical organizations and regulatory agencies. Most are direct colorimetric measurements based on the application of nitrate reduction, nitrite diazotization, and subsequent azo coupling to form strongly colored azo compounds then measured at levels presumably low enough for global regulatory requirements. For complex food matrices, especially high protein dairy products, these methods struggle to provide the required sensitivity and selectivity to meet global regulatory guidelines. To address these issues a method validation study has been conducted utilizing high performance ion chromatography with Greiss-reagent-based post-column derivatization for the determination of nitrite and nitrate in lactose, milk powders, and whey protein concentrates. This comprehensive validation addresses all aspects of method performance for this HPIC-PCD-based method including linearity, specificity, sensitivity, accuracy, repeatability, reproducibility, and robustness. The method has been demonstrated to provide linearity over multiple orders of magnitude, detection limits of 10-100 ppb, typical in-matrix spike-recovery of 80-120% with precision, and robustness necessary to be utilized as quality assurance tool to ensure high protein dairy products meet the most stringent global regulatory requirements.

Recent Advances in Suppression Technology for Ion Chromatography

The role of a suppressor in Ion Chromatography is well accepted. When pursuing anion analysis the purpose of the suppressor is to remove the cation components of the eluent and the sample and replace it with hydronium ions. The net effect is formation of a weakly dissociated suppressed eluent product and for fully dissociated analyte ions conversion to the fully dissociated acid form thus increasing the signal to noise. Supply of the regenerant ions for the function of suppression is achieved in the electrolytic suppressor from the electrolysis reactions. Typically the suppressor is operated in the constant current mode and the required current for suppression is computed using some equations that are based on Faraday’s law that correlate the eluent concentration to the current for a given flow rate. With current efficient suppressor devices by operating them with constant voltage the current required for suppression automatically adjusts to the influent eluent concentration. In this presentation we discuss the design of devices that operate under constant voltage conditions. The benefit of the constant voltage devices will be demonstrated here for gradient applications. Applications to real life samples such as drinking water samples will also be presented using the current efficient suppressor devices.

Keywords: Environmental Analysis, HPLC, Instrumentation, Ion Chromatography
Application Code: Environmental
Methodology Code: Liquid Chromatography
The expansion of legalized marijuana, both for medical as well as recreational use, has led to an increase in analytical testing needs such as potency, pesticides, terpenes, and residual solvents. And while the murkiness of the regulatory landscape may be at the heart of many of the challenges, market driven solutions are on the rise, particularly in labs with parallel areas of expertise. Challenges in pesticide testing often arise from a wide range of physical and chemical analyte properties in multiresidue screening along with diverse sample matrix interferences. Sample clean-up with QuEChERS has become widely adopted in food matrices to remove matrix interferences. Alternatively, a dilute and shoot approach might be taken when sensitivity isn’t compromised, such as with high sensitive mass spec detection. Both approaches have been implemented successfully for pesticides in cannabis sample testing, which we demonstrate on multiple and complimentary LC column chemistries in HPLC and UHPLC platforms for the 64 pesticides indicated from the Oregon Health Authority. Luna® Omega Polar C18 and Kinetex® Core-Shell Biphenyl yield excellent retention and peak shape for the challenging and earliest eluting daminozide, while maintaining a solid chromatographic profile throughout the range. Similar challenges for potency determination of the dozen or so cannabinoids of interest also persist, given the diverse sample matrices, levels of cannabinoids in various strains, and workflows in play. These diverse selectivity options on highly efficient and versatile platforms can be an excellent tool to navigate the myriad of established matrix interferences and those to arise.

Keywords: HPLC, HPLC Columns, Liquid Chromatography/Mass Spectroscopy
Application Code: General Interest
Methodology Code: Liquid Chromatography
Some Hint on How to Make a Standard UHPLC Column with + 300 000 Theoretical Plates/Meter

The race to achieve the highest number of theoretical plate counts has been going for many years already, even though it could and should be argued that a good separation is mainly governed by good selectivity, a reasonable retention and then high column efficiency. However, the race has still been going, and this presentation will show how + 300 000 theoretical plates/meter can be achieved in a standard column for UHPLC. The importance of a good bonding and end capping technique will be discussed and hints about how this is made is revealed. The importance of base particle choice with a good heat transfer capacity will be shown and the importance of small extra column peak broadening will be emphasized. The + 300 000 theoretical plates/meter is shown in two different columns lengths with the inner diameter of 2.1 mm, 150 and 50 mm long respectively.

Keywords: HPLC, HPLC Columns
Application Code: High-Throughput Chemical Analysis
Methodology Code: Liquid Chromatography
It has been well known since publishing the paper by Nagae [1,2] that a mobile phase was expelled from the pore of packing materials for reversed phase liquid chromatography under an aqueous condition by capillarity, consequently, retention decreased. Capillarity depends on the contact angle of a liquid on the surface of a substance. In the case of more than 90 degree of the contact angle, in other words, non-wetting, the force brought by capillarity makes a liquid expel from the pore. Reversely in the case of less than 90 degree of the contact angle, in other words, wetting, the force brought by capillarity makes a liquid permeate onto the pore. In this study, it was revealed that even if the contact angle was more than 90 degree, a liquid was not always expelled from the pore. Only when the contact angle was less than 90 degree, and furthermore the force brought by capillarity was more than the atmospheric pressure, a liquid was expelled from the pore. Permeating or depermeating (expelling) of a mobile phase from the pore doesn’t always depend on wetting or non-wetting of a liquid on the surface of packing materials. The expression of dewetting has been often used when a mobile phase was expelled from the pore of packing materials and retention reduced. This expression, however, was considered not to be factual. It was suggested that the expression of depermeating should be used in such a case.
Simultaneous Estimation of Ketorolac Tromethamine and Phenylephrine Hydrochloride in Artificial Aqueous Humor

Ketorolac Tromethamine and Phenylephrine Hydrochloride Injection was approved for use during cataract surgery. To determine concentration of both drugs simultaneously, in aqueous humor, no analytical chromatographic method was reported in literature. Present research describes validated Reverse Phase High Performance Liquid Chromatography method for estimation of Ketorolac Tromethamine and Phenylephrine Hydrochloride in artificial aqueous humor. HPLC method was developed using C18 (250 mm × 4.6 mm id, 5 µm) column using Methanol: Water (0.5% TEA, pH 6 adjusted with 10% glacial acetic acid) as a mobile phase. Run time of the method is not more than 8 minutes. Validation was performed according to USFDA guideline. The calibration curve was linear over the range of 0.09357-12 µg/ml for Phenylephrine Hydrochloride and 0.0625-8 µg/ml for Ketorolac Tromethamine with correlation coefficient near to 1. Lower Limit of Quantification is 0.09357 µg/ml and 0.0625 µg/ml for Phenylephrine Hydrochloride and Ketorolac Tromethamine respectively. Protein precipitation technique was used as sample preparation method and mean extraction recoveries were found within 95.02-95.73%. Accuracy was found to be 98.49-100% and 99.92-100.07% with coefficient of variation 0.7537-0.8879% and 0.8240-1.2648 % for Phenylephrine Hydrochloride and Ketorolac Tromethamine respectively. All validation parameters were complied to the acceptance criteria as per USFDA guideline.

Keywords: Bioanalytical, Drugs, HPLC, Pharmaceutical
Application Code: Bioanalytical
Methodology Code: Liquid Chromatography
Many of the top-selling pharmaceuticals currently on the market and in the pipeline are biologics. Because biologics are more complex than small molecules, analytical methods for analysis and regulatory requirements tend to be less straightforward. To date, many of the methods used in development and quality control laboratories are HPLC-based. While this may be sufficient in some cases, there are noted advantages of updating legacy systems and methods with more modern instrumentation. Regulators also recognize and support the notion for improving process performance through lifecycle management in an effort to enhance product quality and patient safety.

The product lifecycle includes development and manufacturing activities as well as technology transfers. When adopting new technology, it is of critical importance that instrumentation be robust and easily deployed. In this work, a new-to-market dual flow path biocompatible UHPLC platform will be used to demonstrate the benefits of laboratory modernization in support of lifecycle management. An HPLC method for peptide mapping is scaled to demonstrate equivalency across HPLC, UHPLC, and UPLC platforms. By updating from an HPLC platform, better resolution, shorter run time, and narrower peak profiles can be achieved. As analytical assays are transferred to various in-house laboratories and contract organizations, it is imperative that results are consistent among sites. Intersystem reproducibility is shown among three systems and assessed using retention time and peak area percent RSD. Finally, an in-line mass detector will be used to confirm CDR peptides of a monoclonal antibody standard, which adds confidence to results within a biotherapeutic environment.

Keywords: Biopharmaceutical, HPLC, Pharmaceutical, Quadrupole MS
Application Code: Pharmaceutical
Methodology Code: Liquid Chromatography
Abstract Text

Core-shell particles (superficially porous particles, SPP) have recently been proven to achieve ultrafast separations with high efficiency for chiral molecules in HPLC. Compared to fully porous particles (FPP), SPP have a rough topography and narrow particle size distribution that they provided better packed bed homogeneity and lower eddy dispersion, respectively. In addition, the solid core also prevents the diffusion of molecules through the entire diameter of a silica particle, allowing fast mass transfer even at higher flow rates. These factors allow the chromatographer to perform rapid and highly efficient separations with moderate back pressures.

A set of 30 basic chiral molecules from a wide variety of compound classes, including primary, secondary and tertiary amines, were tested for this investigation. Many of these compounds have not been well separated until now. In this study, four different chiral stationary phases (CSPs), core-shell (2.7 $\mu$m) bonded vancomycin, modified-vancomycin, hydroxylated beta-cyclodextrin and isopropylated cyclofructan-6 were utilized for the enantiomeric separation of these analytes. Cyclofructan-6 column has shown to be the most advantageous in separating the majority of primary amines. Two vancomycin-based columns showed powerful chiral selectors for most basic compounds. The beta-cyclodextrin-based CSP also showed good chiral selectivity for basic, aromatic compounds. Furthermore, these CSPs are multi-modal, where they can be operated in polar ionic/polar organic mode and reversed phase mode (except for Cyclofructan-6), which is important for biological samples where mass spectrometry compatibility is needed.

This presentation will demonstrate the power of these four versatile CSPs and the ease of screening and optimization procedures. Details in HPLC parameters will be discussed as well as the improved chromatographic performance afforded by the core-shell silica particles, compared to fully porous particles.

Keywords: Chiral Separations, HPLC, Liquid Chromatography, Pharmaceutical

Application Code: Pharmaceutical

Methodology Code: Liquid Chromatography
Requirements in chromatographic analysis are constantly evolving: lower limits of detection need to be achieved, faster and more confident confirmation of analytes is required, and method development time needs to be reduced. Mass spectrometric detection can meet these requirements but is often considered too complex for chromatography labs. The new Thermo Scientific™ ISQ™ EC single quadrupole mass spectrometer delivers robust performance and is fully integrated into the Thermo Scientific™ Chromeleon™ 7.2 chromatography data system making operation and data analysis straightforward and intuitive. Three applications demonstrate the value of mass spectrometric detection to chromatographers:

1. In medicinal chemistry the success of organic synthesis of new molecules needs to be established. LC-MS analysis results in identification and quantification at the same time. Additionally, it is suitable for high throughput.
2. Impurity analysis of produced pharmaceuticals is essential. According to ICH guidelines all side products above a certain threshold need to be first characterized and later monitored. In quality control, UV-based detection is still the standard but MS detection is gaining acceptance because of lower detection limits and immediate analyte identification by mass confirmation.
3. Method scouting is the first step in developing a new separation method. Testing multiple conditions for a compound mix can become a time-consuming exercise. In contrast to UV detection alone, MS based method scouting inherently identifies the compounds based on their unique mass and isotopic pattern. Consequently, MS based method scouting can be done in a multiplexed fashion resulting in drastically shorter development times.

Keywords:  HPLC, Liquid Chromatography/Mass Spectroscopy, Pharmaceutical, Quadrupole MS
Application Code:  Pharmaceutical
Methodology Code:  Liquid Chromatography/Mass Spectrometry
During the in-use stability study on an oral dosing syringe for dosing a pediatric suspension formulation, an extra impurity peak was found in the chromatograms of the suspension in-use stability samples, and this impurity peak grew along with increasing duration of storing the suspension in the syringe. Further study showed that this impurity peak is not related to the drug, indicating it may be a leachable from the dosing syringe. Extraction using appropriate diluent was carried out on the dosing syringe, and the extract was analyzed by Q-TOF LC/MS. Based on the exact mass and then generated chemical formula, four commercially available compounds are recognized as possible identification (ID) for the leachable. The ID was further narrowed down to one compound, 2-(2-hydroxyethylmercapto)benzothiazole (HMBT), based on the MS/MS data. HMBT standard was analyzed by HPLC and Q-TOF LC/MS, and was confirmed to be the leachable found from the oral dosing syringe.
Propolis is a chemically complex resinous substance collected by honeybees from tree buds, comprising plant exudates, secreted substances from bee metabolism, pollen and waxes. Its chemical composition depends strongly on the plant sources available around the beehive, which have a direct impact in the quality and bioactivity of the propolis. Recent analytical techniques can be applied to separate and quantify polyphenols in extracts of propolis used in raw, pure and nutraceutical preparations. Crude samples were dissolved in ethanol as extracts of propolis, and analyzed by electrospray ionization mass (ESI-MS) and tandem mass spectrometry (ESI-MS/MS) in the negative ion mode. 40 Colombian samples of propolis from different wildlife zones were evaluated. ESI-MS provides characteristic fingerprint mass spectra. Chemometric multivariate analysis statistically demonstrates the reliability of the ESI-MS fingerprinting method for propolis. On-line ESI-MS/MS tandem mass spectrometry of characteristic [M-H](−) ion markers provides an additional dimension of fingerprinting selectivity, while structurally characterizing the ESI-MS marker components of propolis. P-coumaric acid, 3-methoxy-4-hydroxycinnamaldehyde, 2,2-dimethyl-6-carboxyethyl-2H-1-benzopyran, 3-prenyl-4-hydroxycinnamic acid, chrysin, pinocembrin, 3,5-diprenyl-4-hydroxycinnamic acid and dicaffeoylquinic acid. The negative mode ESI-MS fingerprinting method is capable of discerning distinct composition patterns to typify, to screen the sample origin and to reveal characteristic details of the more polar and acidic chemical components of propolis samples collected at the different wildlife zones in Colombia.

Keywords: Bioanalytical, Chemical Ionization MS, Chromatography, Electrospray
Application Code: Pharmaceutical
Methodology Code: Liquid Chromatography/Mass Spectrometry
The polysaccharides derivatives coated and immobilized on silica are known to be useful for separation of a wide range of racemic compounds. Among these, immobilized type chiral stationary phase (CSP) columns are becoming common for measuring the optical purity, and isolating chiral materials.

We developed a novel immobilized type CSP named "CHIRAL ART Cellulose-SJ" which was cellulose tris(4-methylbenzoate) immobilized on silica particle. Cellulose-SJ has a good feature for the durability under acidic and basic conditions, and under many kinds of organic solvents. Also it has the structural feature of possessing ester linkage in the selector. This leads to different separation in comparison of other carbamate-linked polysaccharide columns. By adding new Cellulose-SJ, increase of hit ratio for the optical resolution is obtained.
Oligonucleotide therapeutics such as antisense, siRNA, aptamer, etc. are expected as next-generation pharmaceutical products following antibody drugs. Reversed phase ion-pair chromatography is appropriate for analysis of oligonucleotide therapeutics and these metabolites. It is difficult to retain and separate highly polar compounds like short oligonucleotides on the ordinary reversed-phase columns. We have a silica-based C18 column named Hydrosphere C18, which provides strong retention of polar compounds and excellent peak shape.

HPLC technique that is utilizing high temperature to generate single-stranded RNA is called “Denaturing HPLC”, and widely used in the field of gene mutation analysis. YMC-Triart C18 using inorganic/organic hybrid silica offers excellent durability at elevated temperature. Both of Hydrosphere C18 and YMC-Triart C18 can be used with a 100% aqueous mobile phase and ideal for oligonucleotides analysis. In this poster, we will be introduced an efficient analytical method for short oligonucleotides using Hydrosphere C18 with an ion-pairing buffer and high temperature analysis of oligonucleotides using YMC-Triart C18.

Keywords: HPLC, Nucleic Acids, Pharmaceutical
Application Code: Pharmaceutical
Methodology Code: Liquid Chromatography
Simultaneous Determination of Multiple Antibiotics in organic Fertilizers Using Ultra Performance Liquid Chromatograph-Tandem Mass Spectrometry

Introduction

It has been reported that organic fertilizers have been widely used in agricultural production. However, it is difficult to remove antibiotics remained in the organic fertilizers, especially tetracyclines, which are the most commonly antibiotics in the manures and soils. In the long run, the residual status of antibiotics will be harmful to human beings, even to leading acute diseases. Therefore, it is imperative to establish efficacy methods to detect the concentration of antibiotics in organic fertilizers to evaluate possible biological risks.

Methods

In the present study, a rapid, sensitive and specific method was developed to quantitatively analyze 15 antibiotics, including penicillins, sulfonamides, tetracyclines and quinolones in organic fertilizers with ultra-performance liquid chromatograph-tandem mass spectrometry (LC-MS/MS). The antibiotics residues were extracted with 80% methanol from organic fertilizers, cleaned up using an HLB SPE cartridge and evaporated to dryness under a rotary evaporator. Then the residues were dissolved in 1 mL of mobile phase and the separation was performed on a Shim-pack XR-ODS III (2.0 mm I.D.×75 mm L., 1.6 μm) by gradient elution with a flow rate of 0.3 mL/min at 40 °C. The analysis was detected with LCMS-8045 under positive and negative electrospray ionization modes at the same time and the quantitation was performed using multiple reaction monitoring (MRM).

Results & Discussion

The results showed that good linearities were obtained with the relative coefficients of the 15 antibiotics standard solutions greater than 0.995. The inter-day precision (RSD) of retention time and area across three concentrations were 0.01~0.46% and 0.29~7.09%, respectively. The recovery rates in the range of 82.2~104.8% were achieved with added concentration as 0.5 and 5 ng/mL. All this figures demonstrated a good suitability of this method for simultaneous determination of multiple antibiotics residues in organic fertilizers.

Keywords: Liquid Chromatography/Mass Spectroscopy, Pharmaceutical, Tandem Mass Spec
Application Code: Pharmaceutical
Methodology Code: Liquid Chromatography/Mass Spectrometry
SFC is a green chromatographic technique that can provide high resolution separations for both analytical and preparative applications. However, many classes of compounds are difficult to chromatograph without the use of additives such as trimethylamine or diethyl amine. This is especially true for mixtures of amines. It would be desirable to chromatograph amine mixtures without the use of additives. At ES Industries we have developed an imidazole based stationary phase that provides superior peak performance for amine containing compounds. We will present several examples using this imidazole based stationary phase for the separation of amine mixtures. We will also present comparison data on commonly used SFC stationary phases using the selected amine mixtures.
Compound assessment of apparent permeability by supercritical fluid chromatography (SFC) is a recent development that can rapidly generate data to help drive the drug design process. The original published method uses a 16 minute chromatographic run for the determination of Exposed Polar Surface Area (EPSA). We have developed a 4 minute method which allows for faster throughput and better instrument compatibility. Examples showing the correlation between EPSA and apparent and intrinsic permeability will also be shown.
Introduction: Levofloxacin is a broad-spectrum antibiotic of the class fluoroquinolone.

Objectives of study: The post market surveillance study on fifteen brands of levofloxacin 500mg tablets registered in Nigeria was aimed at assessment of the physicochemical quality of the brands and determination of the quantity of selected metal impurities. To determine the brands that could be substituted for the innovator brand using similarity factor.

Methodology: The concentration of selected metal (Hg and As) impurities in all the brands of levofloxacin 500 mg (LEV01 to LEV15) tablets were carried out using ICP-OES. The physicochemical parameters of evaluated using both official (USP) and unofficial standards including uniformity of weight test, hardness test, friability test, disintegration test, dissolution profiles and quantitative assay.

Results: The concentration of mercury and arsenic in all the brands analysed is below the Permissible Daily Exposure (PDE). The results showed that all the brands passed the physical tests except Lev 11, Lev 10 and Lev 01, 02, 09, 11 that failed the uniformity of weight test, disintegration test and hardness test respectively. Lev 09 failed the quantitative assay test. Only Lev 3, 6, 7, 8, 11, 13, 15 can be substituted for the innovator brand Lev 01. All the brands manufactured in Nigeria (Lev 6, 11, 13) passed the similarity factor, f2, test and therefore could be substituted for the innovator brand. Conclusion: The study showed that the locally manufactured levofloxacin tablets could be substituted for the innovator brand. The post market surveillance study of medicinal agents is a source of very important information for health practitioners.

Keywords: Analysis, Atomic Absorption, HPLC, Pharmaceutical

Application Code: Pharmaceutical

Methodology Code: Liquid Chromatography
Imp X is a reactant in the process of synthesis of PB-1404 (a Drug Candidate). Per the prediction by ACD/Labs software, Imp X is a potential genotoxic impurity. Thus, Imp X in PB-1404 tablets form should be monitored to ensure drug product safety. As the European Medicines Agency: Guideline on the Limits of Genotoxic Impurities described that a threshold of toxicological concern (TTC) value of 1.5 g/day intake of a genotoxic impurity is considered to be associated with an acceptable risk (excess cancer risk of <1 in 100,000 over a lifetime) for most pharmaceuticals. Therefore, a permitted level which is at ppm in the drug candidate is calculated based on the expected daily dose by this threshold value.

Generally, LC-MS is used to determine genotoxic impurity due to its low detection limit (ppm level). And several HPLC-UV methods have been reported in literatures for the determination of Imp X, however those methods could not be reproduced well at ppm level, i.e. its sensitivity cannot meet the requirement. As HPLC is more universal used than LC-MS under various conditions in pharmaceutical industry due to its satisfactory repeatability and cost-effective characters. A simple, accurate and reliable HPLC-UV method was developed and validated for the quantification of Imp X at ppm level. The Imp X can be well resolved from the interference peaks, which were generated by six kinds of excipients in the tablets form of PB-1404 under 210 nm detection wavelength (the maximum UV absorption of Imp X).

In conclusion, this poster presents method development, validation and results for sample analysis of several batch. Furthermore, there is also great potential to expand this method to determine other genotoxic impurities at low concentration in pharmaceutical industry.
Kramers model that introduced Fokker-Planck stochastic differential equation for the velocity of chemical reactions was based on concepts of equilibrium and escape of a particle through some potential barrier. Fokker-Planck stochastic differential equation was applied recently to Hodgkin-Huxley ion channel model and extended to the different noise models in different sensors that are sensitive to temperature change, kinetics, and outer dynamics. This application also employs concept of equilibrium. Therefore, application of Fokker-Planck stochastic differential equation can be extended to the rate of transcription or translation controlled by riboswitches. Multidimensional time model for probability cumulative function along with multi-scale time analysis can be further applied to the analysis of the rate of transcription or translation. Multidimensional time model for probability cumulative function can be reduced to finite-dimensional time model, which can be characterized by Boolean algebra for operations over events and their probabilities and index set for reduction of infinite dimensional time model to finite number of dimensions of time model considering also the fractal-dimensional time arising from alike supersymmetrical properties of probability. It is based on the properties of composition of Brownian motion processes applied through application of Boolean prime ideal theorem and Stone duality. This model through cumulant analysis, theory of associated random variables, and time series analysis can be extended to uncertainty quantification of complex computational models in different areas of sciences that can require calculation of 5-fold integrals and the like.

Keywords: Bioanalytical, Biosensors, Immunoassay, Nucleic Acids
Application Code: Bioanalytical
Methodology Code: New Method
Dinaciclib (MK-7965, SCH727965) is a cyclin-dependent kinase inhibitor. It inhibits CDK1, CDK2, CDK5, CDK9 and CDK12 in nanomolar range. In phase I trials, dinaciclib demonstrated acceptable cytotoxicity. Dinaciclib recently entered Phase III clinical trial for the treatment of leukemia. The results of the study show its promising antileukemia activity and a tolerability. Dinaciclib causes apoptosis to several cancer cell lines, including those that are resistant to anthracyclines. It is well known that increased metabolism of anthracyclines into their secondary C13-hydroxy metabolites is one of the main mechanisms leading to cancer resistance. To date, the anthraccline reductive metabolism has been associated with members of short-chain dehydrogenase/reductase superfamily (SDR) and of aldo-keto reductase superfamily (AKR). Our results have shown, that dinaciclib is a strong inhibitor of aldo-keto reductase 1C3 (AKR1C3). AKR1C3 is overexpressed in many cancer cell lines and is involved in cancer resistance to anthracyclines. In our study, dinaciclib inhibited AKR1C3 in the experiments with purified recombinant enzyme (IC50 = 235 nM, Ki = 165 nM). The experiments with HCT116 human cancer cells overexpressing AKR1C3 have confirmed that dinaciclib is equally active at the cellular level (IC50 = 200 nM). AKR1C3 inhibitors are strategic compounds for the treatment of cancer. The enzyme is upregulated after pre-incubation of daunorubicin and doxorubicin and its increased metabolism contributes to the pharmacokinetic resistance to anthracyclines in tumor tissues. Our results indicate that dinaciclib may potentially increase the therapeutic efficacy of anthracyclines, prevent anthracycline resistance and minimize their adverse effects. A UHPLC (Agilent) with fluorescent detection was employed to separate and measure concentrations of anthracyclines and their metabolites in all biological samples.

Keywords: Chromatography, Drugs, Enzyme Assays, Pharmaceutical
Application Code: Pharmaceutical
Methodology Code: Liquid Chromatography
Cyano acetic acid is employed as a precursor in the synthesis of many drugs, and it is also a potential hydrolysis degradant of PB-1507 (a drug candidate) in the tablet form. Generally, cyano acetic acid can be determined by standard titration method. However, it may be over-estimated due to the interference of common excipients. HPLC is a popular quantitative analysis technique in pharmaceutical field as it is considered accurate and reliable, whereas it has not been reported for determination of cyano acetic acid in the dosage form in literatures with HPLC method.

Owing to cyano acetic acid’s low molecular weight and high polarity, high aqueous eluents and a specified column should be essential for developing a HPLC method. Meanwhile, cyano acetic acid only has end absorption with no characteristic absorption by UV scan, and shows very low response in UV absorption. Therefore it is challenging to meet the sensitivity requirement for analysis of cyano acetic acid impurity in dosage form using a HPLC method. Besides, when UV end wavelength is selected as the detection wavelength, the interference from excipients in dosage form may be an issue in liquid chromatographic separation. Herein, a simple, rapid, specific and accurate reversed phase HPLC method with UV detection was developed for quantification of cyano acetic acid impurity in PB-1507 tablet form.

A Welch Ultimate AQ-C18, 5\textmu m, 4.6 x250mm analytical column was adopted for the chromatographic separation with the column temperature at 40\degree C. Eluents were A=20 mM Potassium Dihydrogen Phosphate (adjusted with phosphoric acid to pH 2.7)/Methanol (100:1)) and B=MeOH with a flow rate of 0.8 ml/min, and 205 nm was selected as detection wavelength. The results exhibited exceptional linearity, sensitivity, precision and accuracy for determination of cyano acetic acid. We suppose this method could provide a platform for analysis of a large number of organic acids and other high polar molecules in dosage form.
Investigation of the tissue distribution of monoclonal antibodies (mAb) is critical for PK studies but remains a daunting challenge even for LC/MS-based methods. The challenges are associated with the ultra-low tissue levels and the lack of appropriate strategies for sample treatments (e.g. protein extraction and blood removal) that are important to achieve high quantitative accuracy and robustness. Here we address these issues and described a streamlined, novel strategy for highly sensitive and accurate quantification of mAb in various tissues. And for the first time, we established the critical parameters necessary to extract, prepare, and quantify mAb from tissues. This study for the first time demonstrated that mAb in tissues presents at two distinct forms: the free form (~30-60% in various tissues) that can be easily extracted by PBS and the bound form (~40-70%) that associated with insoluble components (e.g. membranes or organelles). Therefore, despite that mAb is a highly polar protein, a strong, detergent-cocktail buffer was found necessary for extraction (>95% efficiency). The novel differential-injection method allowed accurate calculation of blood removal efficiency and positive biases introduced by residual blood. Based on the results of this evaluation, a perfusion strategy using ~3 body-volume of heparinized saline was determined optimal, where >99% of residual blood can be removed without perceivable loss of tissue-associated mAb and positive biases <0.5% were achieved. Using the trapping-micro-LC/MS developed in our lab, low LOD and LOQ of 15 and 48 ng/g-tissue were obtained across seven different tissues, without any affinity enrichment. The time courses of the mAb in plasma and 7 tissues (kidney, liver, lung, spleen, heart, muscle, and brain) were measured in the animal model (single I.V. dosing). It was highly interesting to observe delayed Cmax of the mAb (by 2-8 hrs) in heart, muscle and brain, but not in other tissues.
In recent years, an issue of great interest in drug formulation methods is the relatively poor water solubility of a large fraction of pharmaceutical candidates. The formulation of poorly water-soluble weak acids or weak bases into salts is known to increase the water solubility, bioavailability, and dissolution rate of the active pharmaceutical ingredients (APIs). However, due to processing conditions or reactions with formulation ingredients, salt forms of APIs have a propensity to revert back to the less soluble, unionized form/free base. Therefore, salt disproportionation is extremely undesirable as this process will influence solid-state properties resulting in a physical form with suboptimal physicochemical stability. Understanding how excipients influence the role of salt disproportionation at low drug loadings (1%) is thus of paramount importance.

In this presentation, I will present how we employ stimulated Raman scattering (SRS) microscopy to analyze salt disproportionation for low drug loadings within a multicomponent tablet matrix. We wish to exploit the acquisition speed, sensitivity, and chemical mapping characteristics of SRS to reduce the time and overall expense required to provide earlier feedback for new drug approval. This label-free chemical imaging tool will help formulation scientists detect and understand salt disproportionation and in situ drug-excipients compatibility issues in low dose solid dosage formulations.

**Abstract Text**

In recent years, an issue of great interest in drug formulation methods is the relatively poor water solubility of a large fraction of pharmaceutical candidates. The formulation of poorly water-soluble weak acids or weak bases into salts is known to increase the water solubility, bioavailability, and dissolution rate of the active pharmaceutical ingredients (APIs). However, due to processing conditions or reactions with formulation ingredients, salt forms of APIs have a propensity to revert back to the less soluble, unionized form/free base. Therefore, salt disproportionation is extremely undesirable as this process will influence solid-state properties resulting in a physical form with suboptimal physicochemical stability. Understanding how excipients influence the role of salt disproportionation at low drug loadings (1%) is thus of paramount importance.

In this presentation, I will present how we employ stimulated Raman scattering (SRS) microscopy to analyze salt disproportionation for low drug loadings within a multicomponent tablet matrix. We wish to exploit the acquisition speed, sensitivity, and chemical mapping characteristics of SRS to reduce the time and overall expense required to provide earlier feedback for new drug approval. This label-free chemical imaging tool will help formulation scientists detect and understand salt disproportionation and in situ drug-excipients compatibility issues in low dose solid dosage formulations.
Abstract Text
The matrix in fermentation tanks is difficult to analyze because of the continuous changing of the matrix composition and its complexity. As a result, numerous tests must be run to fully understand the fermentation process. In the production environment, HPLC analysis and PCR provide information hours after the sample is pulled, preventing real-time optimization of the production parameters. Online near-infrared (NIR) spectroscopy can overcome these time hurdles, providing results in real-time through cross-validation with traditional analysis methods. We demonstrate this capability with a biofermentation case study showing real-time monitoring of multiple parameters including sugar species concentration that are important for optimizing process yields.
Background. This research focuses on the effect of various formulation parameters on targeting human epidermal growth factor receptor-2 (HER2), specifically in breast cancer. Poly (D, L-lactide-co-glycolide) (PLGA) polymer, which is approved by FDA and used to form nanoparticles (NPs) encapsulating docetaxel (DOC) as chemotherapy. The HER2 antibody was decorated on the PLGA NPs, as either whole IgG (TrAb) or fragments (ScFv), and investigated regarding their ability to target HER2 breast cancer cells.

Methods. A solvent evaporation technique was adapted to design NP formulations. Incorporation of ligands (TrAb or ScFv) was conducted through chemical conjugation processes. Executing the physicochemical characterization of formulations. So as performing Fourier transform infrared spectroscopy (FTIR) to assess the attachment of different ligands. Also, fully validated mass spectrometry analysis method was used to quantify the loading of DOC. In vitro drug targeting assessed by performing Fluorescence-activated cell sorting (FACS) and western blot.

Results. Particle size was measured to be below 400 nm for the modified PLGA NPs with approximately neutral zeta potential. Encapsulation efficiency for DOC reached up to 85% for some formulations, and the amount of anti-HER2 attachment efficiency exceeded 40%. The cellular targeting of nanoparticles was studied using two cell lines (MCF-7 and SK-BR-3), which express different levels of HER2. The significant reduction in the level of HER2 expression was observed for modified NPs in HER2 overexpressed SKBR-3 cells.

Conclusion. Our data demonstrated a prospective potentiality for this NPs against different cell lines. Thus, ligand modified structurally concealed PLGA NPs could be a promising delivery tool for targeting HER2 breast tumor in vitro that improves the release of chemotherapy while reducing the side effects.

Keywords: Drug Discovery, FTIR, Particle Size and Distribution, Polymers & Plastics
Application Code: Pharmaceutical
Methodology Code: Mass Spectrometry
In recent years, nitric oxide (NO) has gained much attention due to its potential as a therapeutic antimicrobial and anti-inflammatory agent. In particular, the application of NO-release materials for the topical treatments of dermal conditions has been explored, including for foot ulcers in diabetic patients. Beyond its potent antimicrobial and anti-inflammatory properties, NO is known to promote angiogenesis, i.e. proliferation of new blood vessels (for potential wound healing applications). Hence, the preparation of safe and effective topical NO-releasing creams could result in improved methods for the treatment of wounds as well as dermal conditions such as acne.

In this presentation, recent efforts to characterize and quantify the NO-release rates of NO from topical creams containing S-nitrosoglutathione (GSNO), an endogenous species found in blood, will be reported. It will be shown that the NO-release rates from thin layers (e.g., 0.5 mm thickness on a glass substrate) of certain creams/ointments containing GSNO can be measured reliably using either a NO chemiluminescence analyzer or a novel gas phase electrochemical sensor when the headspace over the films is purged with nitrogen gas. Excellent correlation between these two analytical measurements methods will be demonstrated. Another objective is to create stabilized GSNO within a hydrophobic cream/ointment formulation that prevents GSNO degradation during long-term storage and then to release NO upon introduction of a secondary cream containing an accelerant agent that initiates NO-release from GSNO. Different accelerants have been studied, including those that act as catalysts or as reactants with GSNO to release NO. Antimicrobial studies conducted with films of the optimal NO release creams will be shown. Results exhibit potent efficacy against several organisms associated with acne as well as infections within skin wound sites.

Funding provided by the Beijing Institute of Collaborative Innovation (BICI).

Keywords: Biomedical, Biopharmaceutical, Chemiluminescence, Sensors
Application Code: Pharmaceutical
Methodology Code: Fluorescence/Luminescence
Handheld Raman spectroscopy holds the promise of eliminating sample quarantine and statistical sampling for raw material identification (RMID) applications and enabling 100% container verification. However, the variety of packaging materials and types of analytes can cause difficulty for many handheld instruments. Some of the difficulties are very long acquisition or library building times, and sample or packaging material degradation. In this work, we examine common excipients and active pharmaceutical ingredients (APIs) inside a variety of common packaging materials with the MIRA M-3. We show that these difficulties can be eliminated through complete control over the data collection and analysis method combined with orbital raster scanning.
A Simple and Precise BIA-Amperometric Method to Follow the Degradation Process of Pharmaceutical Products

The development of new drugs is a constant necessity in society. However, the rate and conditions in which each substance degrades can vary, resulting in altered efficacy or toxicological effects. Therefore, rigorous tests of drug safety and factors that influence the degradation of each substance are of great importance. Most of the official tests for those substances are based on techniques such as HPLC, which requires organic solvents and high cost equipment. Searching for a simple and effective method, this work describes an alternative for the realization of drug forced degradation tests in a simple amperometric coupled batch injection analysis (BIA) system. The study was carried out in a 4 mL BIA cell, which was designed and printed in 3D, in ABS polymer. The amperometric sensor used in the measurements is based in a nanostructured double hydroxide of nickel and lead (where the proportion Ni/Pb = 3) synthetized by sol-gel methodology and characterized by X-ray diffractometry and cyclic voltammetry. This material was applied on the surface of a graphite sheet, which was used as the working electrode. This system was used to detect the changes of concentration due to degradation of a 5 x 10^{-4} mol L^{-1} solution of L-dopamine in alkaline condition in the presence and absence of oxygen for three hours at 25º C. The study showed that the presence of oxygen greatly accelerated the degradation process in alkaline condition, leading to the complete degradation of the substance by the end of the test. The studies in different conditions, such as acidic medium, thermal and photolytic conditions are still in process.

References:

Acknowledgements:
CNPq, FAPESP and CAPES

Keywords:
Drugs, Electrochemistry, Method Development, Sensors

Application Code:
Pharmaceutical

Methodology Code:
Electrochemistry
Inductively coupled plasma techniques, whether coupled with optical (ICP-OES) or mass spectrometer (ICP-MS) detectors, have been widely adopted in the run up to the implementation of U.S. Pharmacopeial Convention (USP) chapters ‘<232> Elemental Impurities – Limits’ and ‘<232> - Elemental Impurities – on January 1st 2018. For the end user, however, concerned with day to day delivery of data, their primary concern is not related to instrumentation but their ability to provide timely and appropriate results for their analyses via the control software user interface. The software workflow should be straightforward, providing a clear pathway from measurement to reporting of results. The control software should provide advanced features to, for example, eliminate the need for additional manipulation in external software packages that do not provide the required traceability of results. An additional requirement for any software package employed as part of USP <232> and <233> based analyses is to ensure proper management of electronic records compliant with FDA CFR 21 Part 11.

This poster presentation will provide examples of the software features available in the Thermo Scientific Qtegra ISDS Software to assist with the implementation of USP <232> and <233> and the implementation of data management, audit trails and electronic signatures required by FDA CFR 21 Part 11.

Keywords: Elemental Analysis, Pharmaceutical, Software, Trace Analysis
Application Code: Pharmaceutical
Methodology Code: Atomic Spectroscopy/Elemental Analysis
The development of new drug delivery systems has presented challenges for demonstrating bioequivalence (BE) for ANDA submissions. Criteria which are adequate for simpler formulations are insufficient for demonstrating BE using the in vitro option for formulations like emulsions. Viscosity, osmolality, and in the case of emulsions, globule size distribution must be measured and shown to be equivalent to the reference listed drug product. The drug distribution among different phases provides additional information about the formulation and its effect on drug release and bioavailability. Ultracentrifugation can separate the phases of an emulsion which can be assayed to determine drug distribution, but this process takes several hours and requires > 20k g-force. Here, drug distribution among the phases of a nanoemulsion was determined using ultrafiltration which took less time and did not require as much stress on the drug product; < 10k g-force. The drug product consisted of a contiguous aqueous phase, micelles, and oil globules. Ultrafiltration devices were used to separate the aqueous phase from the drug product and also to separate aqueous phase with micelles from the drug product. This process resulted in a filtrate, containing aqueous phase or aqueous phase with micelles, and a retentate, containing concentrated micelles and oil globules or concentrated oil globules with unperturbed concentration of micelles (Figure 1). The drug concentration in the filtrate of was used along with the overall drug concentration in the drug product to determine how drug was distributed among the phases of the drug product.
Pharmaceutical Analyses

**Synthesis and SAR of Benzylamine Spcomplex**

Benzylamine derivative supported complexes have been synthesized. The complexes were tested in vitro against the MCF-7 cell line, and the 4-fluoro and 4-chloro containing complexes expressed impressive anticancer activities. Their DNA binding nature for a structure activity relationship study was investigated with physicochemical indicators which categorized them as good intercalators for host-guest chemistry. A mechanism for drug efficacy is proposed by analysis of the resultant viscosity and surface tension of PtCl4(BADs)2-DNA solutions named as the drugfriccohesity interaction. The complexes have shown significant antioxidant activity, determined on the basis of free radical scavenging effects due to their terminating action against the reactive specie.

**Keywords:** Agricultural, Drugs, Medical, Pharmaceutical

**Application Code:** Pharmaceutical

**Methodology Code:** Chemical Methods
Pharmaceutical Analyses

Reagent Free Near-Infrared (NIR) Spectroscopic Analysis of Moisture and Structure in Lyophilized Products

The quality, efficacy, and shelf-life of lyophilized pharmaceutical products and ingredients depends on water content. Routine Karl Fischer (KF) titration is the standard method for measuring residual moisture content to monitor manufacturing, ensure samples are within specification, and to optimize the freeze-drying process. However, routine application of this technique is not without drawbacks, most notably the use of hazardous chemicals and the destruction of samples. Near-infrared (NIR) spectroscopy is well-suited to measuring moisture, and can do so without sample preparation or chemical consumption. This talk explores how to best link NIR spectroscopy to both KF and structure measurement results for lyophilized materials and how this method can improve efficiency and tighten product specifications.

Keywords: Biopharmaceutical, Near Infrared, Pharmaceutical, Process Control

Application Code: Pharmaceutical

Methodology Code: Near Infrared
The chiral recognition capabilities of different macrocyclic glycopeptides were evaluated in super-critical and sub-critical fluid chromatography mode and compared to the results obtained from high performance liquid chromatography. The superficially porous particles have shown to provide faster analysis times, low back pressures and higher efficiencies making chiral analysis much faster. Four chiral selectors namely CDShell-RSP, Nicoshell, Teicoshell and Vancoshell were used in separation of multiple classes of narcotics including amphetamines, cathinones and anti-depressants. The speed of separation being one of the major advantages of SFC over HPLC, combining it with the superficially porous particles has made enantiomeric separations immensely fast. Also, SFC being advantageous for preparative separations with easy solute recovery, lot of pharmacological studies can be done on the individual enantiomers of these stimulants. In addition, with tougher EH&S laws SFC seems to the way forward because of the generation of much less toxic wastes.
Pharmaceutical Analyses

Cu (II) Glimepiride Complex: Synthesis, Spectroscopic Studies and Antidiabetic Activity

Glimepiride is a third generation sulfonylurea drug used for the management of type 2 diabetes which lowers blood sugar level by stimulating the release of insulin through pancreatic beta cells and by inducing increased activity of intracellular insulin receptors. Copper complex of glimepiride was synthesized by reaction of glimepiride with copper (II) chloride salt. The metal complex was characterized based on elemental analysis, UV, IR, and 13C NMR spectroscopy. Spectroscopy studies showed that glimepiride behave as a tridentate ligand. The mole ratio of the ligand to metal ion was proposed to be 2:1. The structure of copper complex was assigned as octahedral in which the ligand molecules lies horizontally joining the central metal atom. The metal complex showed remarkable hypoglycemic activity as compared with the parent drug ligand in alloxan induced albino rat. After 8 hours of experimental time, Cu metal complex showed significant reduction in blood sugar level more than glimepiride (Cu glimepiride complex 283.25±10.01 to 161.00±14.63) and (glimepiride drug 258.25±25.38 to 187.75±18.71). The metal complex had eligibility to lower blood glucose for more duration of time even after 21 days of experimental time than glimepiride drug (Cu glimepiride complex 126.25±3.30 to 96.75±1.50), and (glimepiride drug 122.50±2.51 to 100.25±2.87).

Abstract Text

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Keywords: Mass Spectrometry, NMR, Spectroscopy, UV-VIS Absorbance/Luminescence
Application Code: Pharmaceutical
Methodology Code: Magnetic Resonance
This study reports the potential of some novel plant materials which can be used in drug delivery and as pharmaceutical excipients. These biopolymers are characterized by CHNS analysis, HPLC and protein analysis. Results show that these biopolymers are carbohydrate polymers. Their property as drug carrier is studied with the help of SIM-ToF, SEM, Disintegration and Dissolution studies by applying different release models and the best fit model is selected. SEM images show that these polymers have voids and layered structure and are the best for incorporating the drugs molecules into the polymers. Drug loaded SEM and ToF-SIMS spectra help to study the dispersion of drug molecules in a polymeric matrix. Release mechanisms of these biopolymers could be classified as Fickian, non-Fickian or super case-II transport depending on the release of incorporated drug through diffusion, swelling, erosion or a combination. Majority of these materials under study showed non-Fickian release involving a diffusion and swelling mechanism. Drug release by these biopolymer materials showed sustained release so they can be used to make slow drug delivery devices. These biopolymers are also evaluated for their potential as binders in tablets, as film-coating agents and suspending agents in pharmaceutical formulations and it has been found that they are no less than the already employed synthetic pharmaceutical materials.

As a result of this study I have discovered some biopolymers which are biocompatible, have high retention duration within the body, degradable by natural mechanisms, low in price and easily available which can be used to make drug delivery devices and as pharmaceutical excipients.

Keywords: Carbohydrates, HPLC, Pharmaceutical, Thermal Analysis
Application Code: Pharmaceutical
Methodology Code: Surface Analysis/Imaging
Session Title: Pharmaceutical Analyses
Abstract Title: Development and Comparison of Quantitative Methods Using Orthogonal Chromatographic Techniques for the Analysis of Potential Mutagenic Impurities

Primary Author: Jennifer Simeone
Co-Author(s): Patricia McConville, Paula Hong

Abstract Text
There are many steps during the manufacturing process of an active pharmaceutical ingredient (API) where impurities can be introduced. Some of these impurities may be mutagenic, or those that have the potential to interact with DNA and ultimately cause carcinogenicity. Methodologies associated with monitoring API purity levels are often HPLC-UV based. This detection technique frequently does not provide the sensitivity levels needed to detect potential mutagenic impurities at the levels required by regulatory agencies. However, the use of tandem quadrupole mass spectrometry can provide both high sensitivity and specificity for these analytical methods. Additionally, mass spectrometry is known to be suitable for use with both Reversed Phase Liquid Chromatography (RPLC) and Supercritical Fluid Chromatography (SFC) methodologies. To evaluate the use of mass spectrometry with both liquid and supercritical fluid chromatography for the analysis of potential mutagenic impurities, an API and five related impurities specified in the USP monograph were analyzed. Ondansetron is a pharmaceutical used in the prevention of nausea and vomiting, and contains two process impurities that are potentially mutagenic. Quantitative methods for the analysis of ondansetron and five process impurities, including the two potential mutagenic impurities, imidazole and 2-methyl imidazole, were developed using two orthogonal chromatographic methods- reversed phase liquid chromatography and supercritical fluid chromatography, yet both methods employed tandem quadrupole mass detection. Method parameters, such as limit of quantitation, linearity, and run time will be compared between the two orthogonal chromatographic methods to determine the benefits of each technique in the analysis of ondansetron and its potentially mutagenic impurities.

Keywords: Chromatography, Liquid Chromatography/Mass Spectroscopy, Mass Spectrometry, Supercritical Fluid Chromatography
Application Code: Pharmaceutical
Methodology Code: Supercritical Fluid Chromatography
Solubility of Active Pharmaceutical Ingredients (APIs) is an important criteria for enhancing bioavailability as it controls the delivery of the drug at the site of absorption. Cocrystallization method has been developed to enhance the solubility of the APIs having poor oral bioavailability. It is different than salt formation as it involves the formation of hydrogen bond, while salt formation involves the formation of ionic bonds. Ritonavir is an anti-retroviral drug, belongs to BCS class II, which suffers from very poor oral bioavailability because of its low water solubility. Hansen solubility parameter was used to predict the miscibility of ritonavir with selected coformers. Cocrystallization technique was used to synthesize cocrystals of ritonavir with various cocrystal formers. Cocrystallization of ritonavir was performed using various methods like solvent evaporation, wet grinding and antisolvent addition. Differential Scanning Calorimetry (DSC) and X-ray Powder Diffraction (XRPD) techniques were employed to support the formation of cocrystals and to find out the stoichiometric ratios of components of cocrystals. In-vitro dissolution study was performed to evaluate the dissolution rate of synthesized cocrystals of ritonavir. Amongst all selected methods wet grinding method was found to be promising for the formation of cocrystals. Dissolution study revealed an increase in aqueous solubility of the synthesized cocrystals with that of pure ritonavir.

Keywords:   Biopharmaceutical, Dissolution, Drugs, DSC

Application Code:   Pharmaceutical

Methodology Code:   Chemical Methods
Diabetes mellitus is one of the most common chronic diseases that is progressive and should be diagnosed and treated early. Linagliptin (L) and saxagliptin (S) are two oral hypoglycemic drugs of the dipeptidylpeptidase-4 (DPP-4) inhibitors class. DPP-4 inhibitors represent a new therapeutic approach for the treatment of type 2 diabetes. They inhibit the enzyme DPP-4 and increase the levels of insulin produced by the body. Recently, single-tablet combinations of DPP-4 inhibitors with metformin (M) have been released and are indicated for the treatment of diabetic patients who are unable to achieve sufficient glycemic control with M alone. Analysis of these combinations presents a real analytical challenge due to the high difference in the dose ratio of the DPP-4 inhibitors to metformin (1:400). In the present study, a quick, simple and reliable validated CZE method was established for the simultaneous determination of L/S with M in pharmaceutical dosage forms. Successful separation of the drugs by the CZE was achieved in a fused-silica capillary by applying a potential of 25kV at 25°C and hydrodynamic injection by 50mbar for 5s. The selected running buffer consisted of 25mM phosphate buffer of pH 9.5 with PDA detection at 225 and 208nm for L:M mixture and S:M mixture, respectively. Specificity, linearity, precision, accuracy, LOD and LOQ and robustness were established for both mixtures in accordance with International Conference on Harmonization guidelines. Electrophoretic separation was obtained within 7 min. The method was linear in the range of 2–200 μg mL⁻¹ for L and S and 25–2000 μg mL⁻¹ for M (R²>0.9984). The calculated percentage relative error and %RSD values for intra-and inter-day precision studies did not exceed 2%. The proposed method was successfully applied for the analysis of the two mixtures in pharmaceutical preparations and proved to be specific and accurate for the quality control of such drugs in their pharmaceutical dosage forms.
In all over the globe, Green chemistry concepts and its role are great challenges towards the development of biologically and pharmacologically active heterocyclic scaffolds via multi component reactions. These concepts clearly describes new carbon-carbon and carbon-nitrogen bond formations in such name reactions with using important catalyst which are very selective, simple, ecofriendly, high yielding, and describe concepts tailored to generate substances quickly by joining small unites together. The various products obtained from the different name reactions likewise: knoevenagel condensation, Mannich, Hantzsch and Beginelli are the important class of target molecules due to their biological and biological importance. We were encouraged to combine different Heterocyclic moieties with hydroxyl derivatives of phenol, urea/thiourea, semicarbazide, primary/secondary amines, different 1,3-diones, ammonium acetate, malononitrile, ethyl-2-cyanoacetate and cyanoacetamide in a single molecular framework. In addition to this, we have synthesized a library of thiophene, quinoline and indole incorporated highly functionalized molecules via green chemistry approach. All the diversely functionalized molecules were synthesized from commercially available starting materials in one-step reaction gives very good yield using different catalyst. The obtained products were screened for antitubercular, antimicrobial, and cytotoxic activities and some of the single crystals studies were to be discussed.
Pharmaceutical Analyses

Flow Techniques - Tool for Research in Pharmaceutical Area

Different flow techniques (FIA, SIA, SIC, MSA, LOV, etc.) are already well established analytical methods, which are characterised by several factors essential for research or routine analysis, e.g. simplicity of fundamental principles, inexpensive instrumentation, low sample consumption and short analysis time. All flow methods offer several advantages, mainly the instrumental set-up is very flexible and the hydrodynamic variables are easily controlled, thus flow methods are becoming a convenient research tool for pharmaceutical area [1].

Automated flow analytical methods has been recently used as a simple sample preparation step (automation of various extraction techniques) or for long-term monitoring. Flow techniques can automate the analysis and control long-term measurements such as dissolution, liberation or permeation tests of pharmaceuticals. A fully automated system based on the SIA technique connected to the Franz cell (enables to mimic real conditions in the human skin and penetration of the drug through the dermal barrier) can easily monitor on-line the release rate of pharmaceuticals [2].

Sequential injection chromatography (SIC) was firstly introduced in 2003 as a simple alternative of high performance liquid chromatography (HPLC) for fast analysis of relatively simple samples. Implementation of short monolithic chromatographic column into SIA has expanded also to analysis of pharmaceutics [3].

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Keywords: Automation, Flow Injection Analysis, Method Development, Pharmaceutical

Application Code: Pharmaceutical

Methodology Code: Microfluidics/Lab-on-a-Chip
Protein-protein complexes and protein drug aggregation are both important problems that require new separation media. Electrophoresis of proteins in gels is limited by the size of proteins that can readily be resolved because of the limited range of pore sizes. Silica colloidal crystals form nanopores that can be made of any desired size to allow for electrophoresis of large proteins or aggregates. These materials intrinsically avoid trapping of large proteins and can potentially be coupled to mass spectrometry. In this work, bare silica colloidal crystals of varying pore size at low pH are investigated for selectivity, efficiency, and speed in the electrophoresis of proteins and aggregates between 100 and 600 kDa. Pore sizes ranging from 35 nm to 60 nm were used to determine the optimal pore size for these large species. The data were found to behave according to the theory of sieving.

Keywords: Capillary Electrophoresis, Electrophoresis, Pharmaceutical, Protein
Application Code: Pharmaceutical
Methodology Code: Capillary Electrophoresis
Amorphous solid dispersions (ASDs), in which an active pharmaceutical ingredient (API) is maintained in an amorphous state within a polymer matrix, effectively improve the bioavailability of poorly water-soluble APIs. ASDs merely reduce the potential for crystallization without completely eliminating the possibility, and so there remains a need for an analytical method capable of stability testing of ASDs with low detection limits. The low limit of detection afforded by second harmonic generation (SHG) microscopy coupled with image analysis algorithms enabled quantitative modeling of the temperature-dependent crystallization of APIs within ASDs. Here a study of accelerated stability testing on ritonavir, a human immunodeficiency virus (HIV) protease inhibitor has been conducted. Under the condition for accelerated stability testing, ritonavir crystallization kinetics from the completely amorphous solid dispersions were monitored by SHG microscopy. SHG microscopy demonstrated the detection limit for ritonavir crystals as low as 10 ppm(v/v), which is three orders of magnitude lower compared to other methods currently available for ritonavir crystallinity detection in ASDs. The access to early-stage crystallization kinetics, which is beneficial from low detection limits of SHG microscopy, enabled quantitative modeling with the JMAK kinetic model. Also, nucleation and crystal growth rates were independently determined based SHG image analysis.

Keywords: Microscopy, Pharmaceutical, Quantitative
Application Code: Pharmaceutical
Methodology Code: Microscopy
Pharmaceutical Analyses

Application of Two-Dimensional Correlation Spectroscopy on Characterization of Biotherapeutics

Biotherapeutics consist of complex molecules including proteins and their derivatives. The structures of these molecules, including primary, secondary and tertiary structure, are important to the potency and safety of the product. Molecular spectroscopy tools have been used to assess the chemical and structural features for the process development, product characterization, batch to batch comparability, etc. One of the disadvantages in using molecular spectroscopy for large protein-like molecules, such as optical, fluorescence, etc., is their low resolution, which only provides much of overlapped spectral bands. This makes it difficult to interpret the physical and structural natures of the spectrum and its corresponding changes. Two-dimensional correlation (2D Cos) spectroscopy utilizes an external physical or chemical perturbation to generate systematically varying signals and provides a way to enhance the resolution by spreading the overlapped signals into second dimension. In this study, preliminary 2D Cos spectra were obtained for monoclonal antibodies between circular dichroism, emission fluorescence and NMR spectroscopy to illustrate the potential application in biotherapeutics characterization.

| Keywords: | Biopharmaceutical, Biospectroscopy, Biotechnology, Spectroscopy |
| Application Code: | Pharmaceutical |
| Methodology Code: | Molecular Spectroscopy |
Non-ionic surfactants are widely used in hydraulic fracturing, which is an important technology for extracting oil and gas. The shale rock is fractured with 10-20 million liters of water per well, and contains part-per-million levels of non-ionic surfactants as lubricating agents and friction reducers. Because of the possibility for groundwater contamination it is important to identify and fingerprint the water that is returning from the fracturing process or left in the groundwater environment. Time-of-flight mass spectrometry was used to analyze and fingerprint the produced water from three locations in the Denver-Julesburg Basin in northeastern Colorado, USA. Three groups of non-ionic surfactants were identified and accurate mass databases were developed.

Furthermore, a long-term field study (405 days) of a well from the Niobrara formation in the Denver-Julesburg Basin was completed. Characterization of organic chemicals used in hydraulic fracturing and their changes through time, from the pre-injected fracturing fluid to the produced water was conducted. The characterization consisted of a mass balance by dissolved organic carbon (DOC), volatile organic analysis by gas chromatography/mass spectrometry, and nonvolatile organic analysis by liquid chromatography/mass spectrometry. DOC decreased from 1500 mg/L in initial flowback to 200 mg/L in the final produced water. Only ~11% of the injected DOC returned by the end of the study. Nonvolatile organic compounds consisted of polyethylene glycols (PEGs), polypropylene glycols (PPG) and linear alkyl-ethoxylates at mg/L concentrations. The distribution of PEGs, and PPGs and their presence throughout the study illustrate their potential as organic tracers for treatment operations or in the event of an environmental spill. Furthermore, a degradation study for these compounds in groundwater was performed and new degradation products were identified and found to be generated under certain microcosm’s conditions.

**Keywords:** Fuels\Energy\Petrochemical, Liquid Chromatography/Mass Spectroscopy, Time of Flight MS, Total Organic Compounds

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

**Methodology Code:** Liquid Chromatography/Mass Spectrometry
Recently there has been considerable interest in gas well drilling into the Marcellus and Utica shale in the Eastern United States. In states such as Pennsylvania, Ohio and others, there has been a dramatic increase in the development of such wells, but this has not been without controversy. While the gas-drilling industry claims that their processes are completely safe and undamaging to the environment, several environmental groups strongly claim otherwise. Adding to the controversy, the process of drilling and hydraulic fracturing of these wells may use various chemicals that are not without concern. The industrial companies claim to self-disclose their formulations, but this is an overstatement, as the exact chemical compositions are not revealed. If drilling or hydraulic fracturing caused some environmental contamination, it would not be easy to determine the source of the contamination due to the lack of disclosure and oversight of the operators. This results in potentially complex environmental forensics analytical method development and sample analysis to determine if a contamination event has occurred, and who the principle polluter may be.

This presentation will address the sampling, sample preparation and analysis of these materials in an effort to develop the chemical compositional understanding of post-drill and/or post-frac fluids so that source identification and source apportionment may be successful in the event of a release and resulting impact to surface and groundwater. Various sample preparation strategies will be discussed. GCxGC-TOFMS and GCxGC-HRTOFMS will be used as determinative techniques due to the inherent advantages over other possible approaches. Further, the development of advanced data reduction strategies will be highlighted to demonstrate how these complex data files and sets may be reduced, increasing the utility of these analytical techniques.

**Keywords:** Environmental Analysis, Fuels\Energy\Petrochemical, Gas Chromatography, Mass Spectrometry

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

**Methodology Code:** Gas Chromatography/Mass Spectrometry
Unconventional oil and gas development (UD) is a multifaceted process for recovery of fossil fuels from low porosity shale. It involves horizontal drilling, well casing, hydraulic fracturing, resource recovery, and wastewater handling. Over the past several years, the Collaborative Laboratories for Environmental Analysis and Remediation (CLEAR) has worked to refine and apply analytical methods to assess the potential environmental impact of UD. Methods include means for characterizing both chemical and biological constituents. These same methods, as well as others, can also be applied to help develop wastewater treatment technologies. Hydraulic fracturing requires an enormous amount of water, and this need has increased as the industry has learned to drill more and longer wells from each pad site. Further, in some regions, such as the semi-arid Permian Basin in west Texas, as many as 9 barrels of produced water are recovered for each barrel of oil. This creates a large waste stream, that if properly treated could be repurposed for various uses. Communicated will be an overview of the method development to date for water analysis as it pertains to both environmental monitoring and wastewater treatment activities performed by CLEAR.
### Session Title
Advances in Environmental Analytical Chemistry: Impacts on Modern Petroleum Production Monitorin

### Abstract Title
Archaean Biomarkers: Adding These Compounds to Environmental Forensics with GCxGC

### Primary Author
Chris Reddy  
Woods Hole Oceanographic Institution (WHOI)

### Co-Author(s)
David L. Valentine, Robert Nelson

**Abstract Text**

Archaean, the third domain of life on Earth, have unique membrane lipids. As opposed to bilayers formed by eukaryotes and bacteria which provide the ester-linked sterane and hopane biomarkers commonly used for petroleum source identification, archaea form monolayer membranes with lipids that are ether-linked to glycerol with C20–C40 branched isoprenoid alkane moieties spanning the glycerol units at each end (De Rosa et al., 1980). These acyclic isoprenoid lipids can serve as biomarkers for environmental oil spill forensics. Along with this suite of acyclic isoprenoid alkanes there are 2-ring to 4-ring cyclic isoprenoid alkanes (Ventura et al. 2007). Here, we highlight the power of these biomarkers in source identification from the 2015 Refugio oil spill (Santa Barbara, CA), which would only be attainable due to the high resolution and separation power of comprehensive two-dimensional gas chromatography (GCxGC).

### Keywords:
Gas Chromatography/Mass Spectrometry

### Application Code:
Environmental

### Methodology Code:
Gas Chromatography/Mass Spectrometry
Petroleum is an extremely complex mixture consisting of tens or hundreds of thousands of different molecular formulas and an even greater number of structures. Exposure of oil to sunlight in environmental systems results in photochemical reactions, many of which lead to oxygenation of the original oil molecules. These transformations create a wide range of photoproducts that mix with the oil, surrounding water, and air. As a result, the number and complexity of species in the mixture increases, providing additional challenges for analysis of these complex mixtures. Consequently, sophisticated tools are needed to gain insight into the compositional changes that occur due to solar exposure. Gaining this insight is important for understanding and managing oil spills. We have utilized high resolution mass spectrometry to determine molecular formulas present when oil samples were exposed to sunlight in the presence of water and air. The photoproducts were observed in the oil phase, the water phase, the interfacial region between the oil and water, and in the gas phase. Results demonstrated that the full range of original oil structures are susceptible to oxygenation, presumably due to the action of singlet oxygen and hydroxyl radical. Furthermore, oxygenation of photoproducts was observed, and samples became more highly oxygenated with increasing solar exposure. The observed products were initially oil-soluble, but become increasingly water soluble as oxygenation continued. We have also utilized derivatization techniques to identify functional groups within the oxygenated photoproducts. Aldehyde and ketone groups were observed through derivatization with 2,4-dinitrophenylhydrazine followed by low resolution electrospray MS-MS analysis. The combination of derivatization and MS-MS allowed selective analysis of a subset of the complex mixture, thereby reducing the complexity of the observed analytes and facilitating the analysis.
The past decade has seen the growth of technologies to facilitate high throughput screening of chemical reactions. Lewis Acids catalyze many important organic reactions and are routinely used in the development of chemical processes to prepare active pharmaceutical ingredients. This presentation will discuss the development of workflows at Pfizer to conduct high throughput screening of Lewis Acids for chemical process development.
Intensifying the deployment of laboratory automation can accelerate the chemistry, manufacturing, and control (CMC) activities and provide a deeper understanding of the pharmaceutical compounds in development. Laboratory automation, such as the Freeslate Core Module 3 (CM3), increases the throughput by automating sample preparation through liquid and solid dispensing (with integrated balance) as well as heating, cooling, stirring, and vortexing in a single platform. The system is programmable through the LEA software suite enabling the scientist to customize each experimental design. The platform interfaces with analytical instrumentation (eg. HPLC and pH electrodes) to automate data handling by pulling the data from an entire study into a single database. These laboratory automation tools facilitated the design and implementation of solubility and forced degradation screens to rapidly screen therapeutic candidates. An Allotrope Data Model and associated Ontologies has been created for the solubility workflow. A LEA adapter converts the data to the ADF Format which includes data generated by the Freeslate platform (such as composition, LC results and pH) in a 96-microtitre plate format. The conversion of high-throughput data into an open-source framework has the potential to facilitate downstream analytics.
Automation in the Pharmaceutical Research and Development Laboratory

Automation of Forced Degradation Studies

This presentation will describe the design, implementation, and use of an automation tool for conducting forced degradation studies. The automated system was designed for fully automated studies and includes components for automated powder dispensing, accurate weighing, dilution, storage of samples at varying temperatures and humidity, automated time point pulls, analysis, and results reporting. The components of the system are modular in order to add flexibility to the system. The automation has significantly enhanced the efficiency of forced degradation studies. In addition, the system has also been adapted for other experimental designs such as internal reference standard weighing and excipient compatibility studies. This presentation will highlight both the benefits of automation as well as some of the challenges encountered during the design and implementation of the system.

Keywords: Automation
Application Code: Pharmaceutical
Methodology Code: New Method
### Session Title
Automation in the Pharmaceutical Research and Development Laboratory

### Abstract Title
Laboratory Automation for Use in Process Development

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### Abstract Text
At GlaxoSmithKline, automation has become increasingly integrated into the process development of drug candidates. Process development in the pharmaceutical industry typically involves experimental workflows that are cumbersome and complex to execute manually, and therefore requires automation tools that are both flexible and have a wide range of functionality. GSK utilizes automated platforms from Mettler, Unchained Labs, Hamilton, and Chemspeed to execute experiments across many workflows in the process development space including reaction screening, organic solubility for crystallization development, competitive ripening screens, and reaction catalyst screens. This presentation will highlight how automation has been used to generate large, robust data sets that give key insight into process related issues and help accelerate decision making by project teams in the drug development process.

### Keywords
Automation, Pharmaceutical

### Application Code
Pharmaceutical

### Methodology Code
Liquid Chromatography
Automation in the Pharmaceutical Research and Development Laboratory

Automated Reaction Development

The design, development, and implementation of a robust and scalable pharmaceutical manufacturing process is predicated on our ability to understand and predict reaction performance. At Bristol Myers-Squibb, a key enabling technology is the use of automated, parallel experimentation to discover catalysts and reagents, identify optimal processing conditions, and determine the most efficient means to isolate the resulting product. The implementation of arrayed, statistical experimental design coupled with multivariate analysis allow both identification of the operating space and construction of predictive models. Through some select examples, this presentation will highlight key aspects of our reaction array design process producing deep, comprehensive data sets resulting in both understanding of chemical reactivity as well as accelerating development from the lab to the plant.

Keywords: Automation, Laboratory Automation, Pharmaceutical, Robotics
Application Code: Pharmaceutical
Methodology Code: Chemical Methods
Emerging Optical Techniques and the Death of Brute Force Explosives Detection

Eyesafe and Portable Standoff Detection of Hazardous Residues Using Quantum Cascade Laser Arrays

This presentation introduces the spectroscopic concepts and results enabled by arrays of Distributed Feedback (DFB) QCLs, with each element at a slightly different wavelength than its neighbor. In portable optical systems, such as standoff threat detectors and in situ gas analyzers, this increases analyte sensitivity and selectivity by broadening spectral source coverage while also allowing for extremely fast all-electronic wavelength tuning with no moving parts.

This talk will first present the QCL array and its packaging, then move into the description of an integrated prototype standoff detection system, and finally show condensed phase standoff threat detection results from a handheld system from over 1 meter. These data are each compared with legacy contact-based methods to ensure that the technique can be reliably deployed to handheld chemical analysis using suitable chemometric algorithms.

The data show how monolithic and all-electronic tuning enables next-generation spectroscopes that are not only more robust and miniature than those that utilize external cavity-tuned lasers, but that are inherently more stable in terms the shot-to-shot amplitude and wavelength parameters. This enhanced stability increases signal to noise for a given configuration (pathlength, averaging time, concentration, etc...). Some discussion of how to maximize the benefits of high speed, highly reproducible tuning is presented, including detector, preamplifier, and digitization considerations.

Keywords: Detection, Infrared and Raman, Molecular Spectroscopy, Spectrometer
Application Code: Homeland Security/Forensics
Methodology Code: Sensors
Traditional hyperspectral imaging (HSI) detection strategies rely on the collection of a data hypercube with a high degree of spectral resolution. Chemometric processing of this hypercube is typically used to characterize and identify regions of interest within the field of view to determine the presence of explosives or other materials of interest. However, this processing must take place after the data is collected, limiting the ability of the sensor to report detections in real time (i.e. greater than 10 frames/s).

In this talk, ChemImage will report on a novel system for the standoff detection of explosives that overcomes these two impediments to on-the-move detection. This next generation HSI technology, known as Dual Polarization-Conformal Filter (DPCF), is a multivariate hyperspectral imaging methodology that uses a two commercial-grade liquid crystal tunable filters each tuned to simultaneously transmit multiple optical passbands. The electro-optic selection of the two sets of optical passbands is optimized for the discrimination of targets versus complex backgrounds through a training process.

In operation, DPCF is analogous to multivariate optical computing, which implementing a multivariate regression (i.e., supervised) classification in the filter transmission function, reducing the spectral complexity for a specific analyte to two filter states. DPCF uses a polarization beam splitting arrangement to present the output of these two filters to a common detector focal plane. In this way, each read of the detector (snap of the camera) provides both images needed for detection. CISS has demonstrated that processed detection imagery can be produced at 15 frames/s, enabling true on-the-move detection.

Aspects of system design of DPCF explosives detection sensor will be presented along with recent test data that shows the analyte selectivity and speed of detection in both stationary and on the move operation.

Keywords: Imaging, Near Infrared
Application Code: Homeland Security/Forensics
Methodology Code: Molecular Spectroscopy
Emerging Optical Techniques and the Death of Brute Force Explosives Detection

Recent Improvements in a Portable UV Raman Standoff Explosive Detection System

Alakai Defense Systems has recently improved its Portable Raman Improvised Explosive Detection system called PRIED. PRIED performs standoff detection of a wide variety of chemicals at ranges of 0.5-10m (some materials are detectable at 50m). The chemicals of interest in standoff detection are Explosives, Homemade Explosives (HME’s), Chemical Warfare Agents (CWA’s), Narcotics and TIC/TIM’s. This system is designed to detect near trace quantities of materials on a wide variety of substrates. Recent improvements include the ability to detect smaller quantities on highly fluorescent substrates. Data will be presented which shows the systems performance.

Keywords: Raman Spectroscopy
Application Code: Homeland Security/Forensics
Methodology Code: Portable Instruments
High-sensitivity non-contact non-destructive trace explosives detection has been an ongoing challenge. We combined the directionality, broad bandwidth, and coherence properties of lasers in order to develop coherent nonlinear modalities of Raman spectroscopy. Results will be shown for both stimulated Raman scattering (SRS) and coherent anti-Stokes Raman scattering (CARS) implemented from a single broadband femtosecond laser pulse. We find this approach can achieve sub-microgram per cm$^2$ sensitivity and selectivity on a variety of explosives at distances of up to 5 meters. Depending on application restrictions, these methods can be implemented with eye-safe lasers and have sub-second imaging speeds. Recent advances in industrial fiber lasers, pulse shaping technologies, and signal digitizers, will facilitate the design of robust, compact, and affordable coherent nonlinear vibrational sensing systems.

**Keywords:** Raman Spectroscopy, Sensors  
**Application Code:** Homeland Security/Forensics  
**Methodology Code:** Vibrational Spectroscopy
Sophisticated and well-concealed IEDs challenge the detection capabilities of current explosive sensors, particularly in areas away from static checkpoints. This security gap could be filled by using standoff (e.g., optically based) chemical sensors which detect IEDs based on external trace explosive residues. Unfortunately, despite intense interest for over the past 15 years, previous efforts to develop standoff trace explosive detectors have not yet led to deployed capabilities. Crucially, the physical morphology of trace explosive residues present unique detection challenges, a fact that is often overlooked in the sensor engineering, as is the role that chemical “clutter” plays in operational performance. In this talk, the unique challenges of non-contact trace explosive detection are outlined and a method is discussed that incorporates realistic trace explosive residues and background clutter into the technology development process, without the need for expensive prototype development. The method is applied to active LWIR and UV Raman spectroscopy for the specific mission of vehicle screening at short range (<1 m) and their performance is compared. The results predicted system performance and areas where additional research is needed. Finally, this analysis framework is currently being applied to compare operational performance of active LWIR and UV Raman spectroscopy for high-throughput non-contact trace chemical screening of luggage.
The single most enduring need for SERS platforms involves 1) increasing sensitivity and 2) increasing reproducibility. The sensitivity is usually impacted most by the structure and morphology of the metallic nanoparticles/nanostructure, while the reproducibility is impacted by the structure of the substrate. While planar two-dimensional substrates have been very effectively utilized, there is a limitation imposed by the surface area. A SERS platform which uses a three-dimensional substrate offers a significant increase in available surface area which can be used to bind metallic nanoparticles. Gold nanorods with appropriate aspect ratios have been shown to be effective SERS particles. In an attempt to fine tune the sensitivity we have explored the use of bimetallic metal nanorods comprised of a gold core covered by a thin layer of silver. These new nanorod structures can be bound electrostatically to electrospun nanofiber mats to produce a stable, reproducible 3D SERS platform. Relative to uncoated gold nanorods, there is a significant increase in sensitivity, and the 3D mesh shows very good reproducibility in SERS enhancement. Results using these structures to measure Pb and As in aqueous solutions will be shown.

Keywords: Environmental Analysis, Raman Spectroscopy
Application Code: Environmental
Methodology Code: Vibrational Spectroscopy

Surface plasmon coupled emission (SPCE) Raman spectroscopy is utilized to determine the chemical composition and thickness of 10 to 100-nm polymer films, biological cells, and self-assembled monolayers on smooth planar gold surfaces. SPCE is performed by optically coupling a sample/gold substrate to a Weierstrass-type prism in the Kretschmann configuration and scanning the angle of the incident laser under total internal reflection. The simultaneous collection and quantitation of the SPCE cone diameter, the SPCE cone intensity and the full Raman scattering signal radiating from the SPCE cone as a function of incident angle are measured using a single instrument. This provides a multidimensional data set for extracting the sample properties. The signals are modeled by two calculated angle-dependent parameters: (1) three-dimensional finite-difference time-domain calculations of the electric field generated in the sample layer and projected to the far-field, and (2) Fresnel calculations of the reflected light intensity. As the sample thickness increases, there is an increase in the incident angles that produce the SPCE cone and Raman scattering as well as an increase in the SPCE cone diameter. Comparing the polymer thicknesses measured using any of the three SPCE parameters to optical profilometry measurements, the average percent difference measured from five polystyrene thin films is 4%. SPCE Raman spectroscopy is a viable non-destructive method capable of determining chemical composition and thicknesses of 100-nm and thinner films with self-assembled monolayer sensitivity.

This research is supported by the U.S. Department of Energy, Office of Basic Energy Sciences, Division of Chemical Sciences, Geosciences, and Biosciences through the Ames Laboratory. The Ames Laboratory is operated for the U.S. Department of Energy by Iowa State University under Contract No. DE-AC02-07CH11358.

Keywords: Raman Spectroscopy, Vibrational Spectroscopy
Application Code: Polymers and Plastics
Methodology Code: Vibrational Spectroscopy
Illuminating Membrane Receptors via TERS

The unique properties of nanostructured metals are enabling interesting applications in biology and medicine. In particular, localized surface plasmon resonance can be utilized for imaging and trace detection. The plasmonic enhancement of Raman signals by nanostructures provides a sensitive label-free method of chemical analysis. Results we have obtained demonstrate plasmonic interactions between a functionalized nanoparticle and a gold nanoparticle atomic force microscope tip enable highly selective investigations of protein receptors in cellular membranes. This tip enhanced Raman (TERS) experiment obtains chemical, structural, and spatial information simultaneously. We are using this approach to study the receptor – ligand recognition in integrins. Our results show signals characteristic of both the ligand, bound to a nanoparticle probe, and also the targeted integrin protein. The observed signals can differentiate between different integrins. Interestingly, the plasmon resonance that enhances the Raman signal can also be used to track nanoparticle probes interacting with cells. Correlations between particle tracking and spectral variance in TERS experiments suggests a new approach for determining binding sites and ligand specificity for drug discovery.

Keywords: Bioanalytical, Microscopy, Surface Enhanced Raman Spectroscopy
Application Code: Bioanalytical
Methodology Code: Vibrational Spectroscopy

Electron, proton, and group transport reactions in proteins have been probed with vibrational coherence spectroscopy, resonance Raman spectroscopy, and ultrafast kinetic measurements [1-4]. Recent studies have demonstrated how excitations and conformational interconversions associated with “soft” low-frequency vibrational modes can control proton and electron tunneling reaction rates by many orders of magnitude (1-2). Examples involving specific biomolecules will be presented showing how low-frequency modes are utilized to facilitate barrier crossing and tunneling reactions. Studies of heme protein systems demonstrate that impulsively driven Raman vibrational coherence (or vibrational coherence spectroscopy) can be used generally as a sensitive probe of thermally accessible and functionally relevant distortions of the active site heme chromophore. The symmetry forbidden signals involve optically driven coherent motions that become allowed due to protein-induced distortions of the heme along its out-of-plane normal modes. These modes have low frequencies (< k_BT<~300K~200 cm^-1) so they are able to extract energy from the thermal bath and serve as biochemical reaction coordinates. Vibrational mixing with other delocalized low-frequency modes of the protein, or with binding partners, may also offer potentially useful methods for the control of protein function.

REFERENCES

Keywords: Biospectroscopy, Raman Spectroscopy, Ultra Fast Spectroscopy, Vibrational Spectroscopy
Application Code: Biomedical
Methodology Code: Vibrational Spectroscopy
The combination of atomic force microscopy (AFM) and infrared (IR) spectroscopy is a powerful tool that provides chemical and conformational information at a spatial resolution of 50-100 nm. Using an AFM-IR instrument, we have explored the correlation between structure, processing and crystallinity in nanofibers[1], ultrathin films and isothermally grown single crystals of poly[(R)-3-hydroxybutyrate-co-(R)-3-hydroxyhexanoate] (PHBHx), which have been produced with a wide range of crystallinity and molecular orientation. They have been characterized using AFM-IR spectroscopy, IR imaging and TEM in order to assess the role of processing on orientation and morphology. For example, AFM-IR allows direct investigation of both the crystalline and amorphous phases of ultrathin polymer single crystals. It provides IR spectra and AFM images simultaneously of sub-100 nm features. The source is a tunable IR laser whose wavelength can be swept through the infrared “fingerprint” region in less than 1 minute. If one of the wavelengths is absorbed by the sample, then expansion of the sample occurs on the ns time scale, which causes a modulation of the oscillating AFM cantilever. This creates a “ringdown” at that particular frequency which decays as the heat dissipates. The positive amplitude of the oscillation represents the IR band intensity and hence as the frequency is tuned through the IR region (900 cm-1 – 3600 cm-1), an IR spectrum is obtained at a spatial resolutions of 50 - 100 nm. More details of this instrumentation is reported elsewhere[2].


Keywords: Microscopy, Nanotechnology, Polymers & Plastics, Vibrational Spectroscopy
Application Code: Polymers and Plastics
Methodology Code: Vibrational Spectroscopy
Tissue structure is defined as the three dimensional organization of cells and extracellular matrix. Achieving a correct tissue structure in engineered tissues is a prerequisite for their normal function and for their applications in medicine and drug screening. However, it remains challenging to engineer tissues with a correct three dimensional architecture, and in many cases, the specific structure of human tissues remains poorly characterized. I will discuss efforts in my lab to build in vivo-like human tissues by leveraging new technologies and an understanding of tissue's underlying programs of self-organization.
The tumor microenvironment is defined by a series of overlapping chemical gradients. These gradients result from poor vascularization of the rapidly growing cell mass, and are a direct result of cellular consumption and waste generation outpacing the rate of vascular exchange. The invasion of cells from the tumor into surrounding healthy tissue is directed by chemical gradients, and oxygen has long been thought to be a primary player. We have recently shown that oxygen is a chemoattractant in 3D cultures where gradients are a result of cellular consumption. In this talk I will highlight the paper-based culture system our laboratory is using to study cellular invasion in the presence of oxygen gradients. By combining fluorescence microscopy with luminescent oxygen and pH sensors, I will show our efforts to distinguish the types of movement that occur under oxygen gradients of different steepness. Our most recent findings suggest that oxygen gradients formed in our 3D culture platform are responsible for directed (chemotactic) movement.
Imaging-Based Methods to Evaluate Tissues, Tumors, and 3D Tumor Models

Open-Top Light-Sheet Microscopy for Nondestructive Slide-Free 3D Pathology of Clinical Specimens

Pathologic features provide a gold-standard by which diseases are diagnosed, patient prognoses are determined, and treatment decisions are made. In a pathology lab, the examination of a tissue biopsy or a surgically excised specimen is most-reliably performed by the microscopic examination of tissues that have been formalin-fixed and paraffin-embedded, sectioned, stained, and mounted on glass slides. This is a labor- and time-intensive process in which only a fraction of a specimen is sampled. The ability to rapidly image intact tissues at high resolution over large areas or volumetric fields of view, with the same level of morphological and molecular contrast that is possible through conventional pathology, has the potential to improve patient treatments and outcomes. While nondestructive microscopy of clinical specimens has been attempted using a variety of approaches, most prior methods have been slow and difficult to use in a clinical setting. To overcome these limitations, we have designed light-sheet microscopes that feature an inverted “open-top” architecture, enabling large specimens to be conveniently placed on top of a flat glass plate and imaged from underneath. A number of applications have been explored. For example, post-operative surface microscopy of fresh prostatectomy specimens with highly irregular surfaces enables rapid triage-inspection of these specimens. In addition, rapid intraoperative surface microscopy of freshly excised breast tissues may be used to guide lumpectomy procedures. Finally, multi-color volumetric imaging of optically cleared core-needle biopsy specimens has the potential to improve upon the accuracy of traditional slide-based histopathology for the definitive diagnosis and grading of lesions.

Keywords: Biomedical, Fluorescence, Instrumentation, Microscopy
Application Code: Biomedical
Methodology Code: Microscopy
For a patient with advanced colorectal cancer there are limited clinical options aside from chemotherapy. Unfortunately, the development of new chemotherapies is a long and costly process. New methods are needed to identify promising drug candidates earlier in the drug development process and to determine their distribution and metabolism in tumor tissue. Here, we are using a novel in vitro platform to assess the treatment of 3-dimensional colon cancer cell cultures, or spheroids, with combination chemotherapies. We culture colon carcinoma HCT-116 cells with heavy or light SILAC media and grown into multicellular tumor spheroids. We dose these spheroids with a common combination chemotherapy, FOLFIRI (folinic acid, 5-fluorouracil, and irinotecan) in a 3D printed fluidic device. This fluidic device allows for the dynamic treatment of spheroids across a semipermeable membrane. Following dosing, we harvest the spheroids for quantitative proteomic profiling to examine the effects of the combination chemotherapy on the colon cancer cells. We also assess the spheroids for cell viability, and analyze them for drug concentration via LC-MS/MS, and image them for proteins and small molecules with MALDI-Imaging Mass Spectrometry. Beyond traditional drugs, we have also imaged liposomal-based therapeutic configurations and shown that our MALDI-based platform is effective in determining the location and metabolism of liposomal configurations. Finally, we will also discuss the use of nutrient restriction to augment chemotherapeutic efficacy in 3D colon tumor models, as determined by MALDI-imaging mass spectrometry and other analytical methods.
Imaging-Based Methods to Evaluate Tissues, Tumors, and 3D Tumor Models

Development and Application of IR-MALDESI for 2D and 3D Imaging

Mass spectrometry offers the most robust platform to discover and characterize new diagnostic, prognostic, and therapeutic biomarkers for ovarian cancer across all molecular classes. Moreover, a systems biology approach will allow the underlying biology of ovarian cancer to be understood. This presentation will discuss the challenges specific to the study of epithelial ovarian cancer (EOC) in humans and how these challenges have directed our thinking, in terms of the development of model organisms and mass spectrometry-based bioanalytical strategies. First, to augment the human model, we developed the domestic hen model of spontaneous EOC, which allowed us to longitudinally sample the rapid onset and progression of the disease in a controlled environment. Second, we developed a novel ionization technique for tissue imaging of lipids and metabolites. This unique model organism has and continues to provide new insights into the biology of ovarian cancer; combined with other –OMICS data obtained through these novel bioanalytical approaches, we will understand the origin of ovarian cancer and ultimately translate that knowledge to humans. Finally, we have developed IR-MALDESI for both 2D and 3D imaging applications and new data will be presented showing these novel features of the approach.

Keywords: Biological Samples, Biomedical, Biopharmaceutical

Application Code: Biomedical

Methodology Code: Mass Spectrometry
Many powerful attributes of NMR spectroscopy are widely recognized, such as its high reproducibility, non-destructive nature, molecular structural content, and quantitative character. At the same time, there exist several misconceptions among metabolomics practitioners about the suitability of NMR for metabolomics studies. These include that (i) NMR is not very sensitive and allows in typical metabolomics mixtures the observation of only few metabolites, (ii) NMR is very slow, in particular when using multidimensional techniques, and (iii) NMR cannot be easily paired with mass spectrometry for the combined analysis of complex mixtures. I will explain these myths and how they can be addressed based on advances in experimental design, processing algorithms, and analysis methods. I will demonstrate how public web servers, including our own COLMAR server (http://spin.ccic.ohio-state.edu/index.php/colmar), permits the simultaneous analysis of multiple 2D NMR spectra for the accurate identification of large numbers of metabolites, how sparse sampling and advanced spectral reconstruction schemes can shorten the time for a 2D TOCSY experiment to about 15 minutes bringing it close to the typical duration of a 1D 1H experiment, how such spectra can be analyzed by graph-theoretical tools for the identification of entire spin systems of both known and unknown metabolites, and, finally, how NMR can be synergistically combined with modern mass spectrometry and combinatorial cheminformatics for the structure determination of unknowns. These tools will be demonstrated for several applications.

Acknowledgements
This work is supported by the National Institutes of Health.

Keywords: Mass Spectrometry, Metabolomics, Metabonomics, NMR
Application Code: Biomedical
Methodology Code: Magnetic Resonance
We seek to quantify the contribution that different nutrients make to the biomass of a cell. This requires: (i) measuring nutrient fates with high molecular specificity, and (ii) determining the fraction of each molecular fate with respect to the total composition of the cell. Our approach to the problem is to feed cells (or animals) nutrients containing 13C isotopic labels. We then lyophilize the samples and measure them intact with solid-state NMR. Following NMR analysis, we extract metabolites from the samples and perform LC/MS-based metabolomics. It is now routine in LC/MS-based metabolomics to measure thousands of signals from a typical biological sample. After feeding isotopically labeled nutrients to cells or animals, we map nutrient transformations at the comprehensive scale by monitoring LC/MS signals that become isotopically enriched. While LC/MS can identify molecular transformations with high resolution, the fraction of nutrient transformed into each product cannot be easily determined because LC/MS does not measure macromolecules such as proteins, DNA, and glycogen. In contrast to the LC/MS results, solid-state NMR 13C spectra of intact samples contain less than 10 signals. Yet, these signals provide a total accounting of carbon in the cell and relative ratios of major molecular classes. Thus, LC/MS and solid-state NMR are highly synergistic for mapping nutrient fates.
Metabolomics Today: MS vs. NMR?

Standardizing NMR and MS-Based Metabolomics

Both NMR spectroscopy and mass spectrometry have become essential platforms for metabolomics research. Each has their own strengths and weaknesses. However, the protocols used by researchers to identify and quantify metabolites varies tremendously from one lab to another and from one platform to another. Most approaches involve considerable manual effort and “artistic” data processing. The absence of standardization and the lack of consistent, universal protocols means that many published metabolomic results are not easily reproduced nor are they comparable. This is becoming a significant barrier to further advances in the field. In an effort to help resolve these problems, my laboratory has been actively developing automated software tools and standardized “kits” to permit consistent, fully quantitative MS and NMR-based metabolomics. Our intent is to ensure greater consistency and improved data quality both within and between labs. In this presentation I will describe some of the freely available data processing software we have developed along with some of the NMR, LC-MS and GC-MS metabolomics kits we have created. By creating these software tools and sharing the methods we used to create these kits we are hoping to encourage more metabolomics researchers to join in this effort to standardize and improve the reproducibility of metabolomics measurements.

Abstract Text

Bioanalytical, Gas Chromatography/Mass Spectrometry, Liquid Chromatography/Mass Spectroscopy

Application Code: Bioanalytical

Methodology Code: Magnetic Resonance
Due to the chemical diversity of metabolites, maximum metabolome coverage can only be achieved by applying multiple analytical technologies. Here, we use 1H NMR and LC-MS (HILIC, RP, +ve, -ve) to make an a priori prediction of the likelihood of prostate cancer (PCa) recurrence prior to prostatectomy. Such predictions would enable the avoidance of unnecessary surgery and associated complications in patients not likely to be cured by such procedures. HILIC and NMR experiments were designed to provide coverage for polar metabolites, while RP mode experiments provided coverage for lipid metabolites. The most significant alterations associated with recurrence were observed in purine and pyrimidine pathways. Patients without post-operative cancer recurrence also had significantly higher serum glucose prior to surgery while those that recurred had higher pre-operative lactate. About half the triglycerides detected, as well as much of the lipids across all saturations were increased in patients that later recurred. Feature selection from the fused NMR+MS dataset yielded 20 features that distinguished between patients that had recurrence or went into remission following surgery with approximately 94 percent accuracy. Lipids, purines pathway metabolites, and amino acids were among the features that underlie the separation between the two groups. We provide strong evidence that a multiplatform analytical approach provides in-depth views of prostate cancer metabolome alterations and prediction of recurrence from a single pre-operative blood sample.

Keywords: Mass Spectrometry, NMR, Metabolomics, Metabonomics
Application Code: Genomics, Proteomics and Other 'Omic
Methodology Code: Mass Spectrometry
We used 13C and 15N tracers to track atoms from enriched sources into various metabolites that report on central energy-producing, anabolic, and anti-oxidative pathways in human lung cancer cells, 3D spheroids/organoids, and tissues ex vivo. Multi-dimensional NMR experiments were employed to determine the 13C label position in various metabolites directly in crude extracts. These include 2-D 1H TOCSY, 1H[13C]-HSQC, 1H[13C]-HMBC, HCACO, and 13C-13C INADEQUATE. These experiments detect 13C atoms with or without attached protons, providing robust assignment of each metabolite and the positions of 13C enrichment. Parallel analysis of the same samples by ion chromatography coupled with ultra high-resolution FTMS (IC-UHR FTMS) provided the number of 13C and 15N labels in numerous metabolites. The two platforms complement each other to provide detailed labeling patterns (isotopomers and isotopologues) of a large number of metabolites for robust reconstruction of metabolic networks vital to cancer cell proliferation and survival. We found 13C6-glucose and 13C5,15N2-glutamine to be readily transformed into various metabolites with labeling patterns distinct for lung cancer cells and tissues, which enabled the reconstruction of reprogrammed metabolic pathways in response to cancer development and anti-cancer agents. These included glycolysis, canonical and anaplerotic Krebs cycle, serine-glycine-one carbon metabolism, purine/pyrimidine/sugar nucleotide synthesis, glutathione metabolism, glycogen turnover, and lipid metabolism. We have also employed chemoselective derivatization approach to enrich carbonylated metabolites with 15N for 1H[15N]-HSQC analysis of these low-abundant and labile metabolites.
The field of Metallomics is arguably less than two decades old. In that time, at least one journal has appeared that is dedicated to and named after it, a biennial international conference on the subject has been established, and sessions on Metallomics can be found in most important conferences on analytical chemistry. In the earlier days of the evolution of this relatively young field, a strong emphasis could be found in manuscripts and lectures on the development of novel tools that meet the very stringent needs of metallomics analysis: extensive elemental coverage, nearly incredible dynamic concentration range (~10^14), and applicability to many different sample types. In addition, editorial boards and scientific sessions invariably included many prominent scientists who specialized in analytical chemistry and the development of novel instrumentation. In recent years, this situation has changed; few seem engaged in expanding the toolbox for metallomics; instead, the field appears to have moved toward bioinorganic chemistry, clinical chemistry, and environmental science, where the emphasis is on application of existing commercial instrumentation. Natural questions, then, are whether there is a need for additional capability, whether tool-builders have strayed from the field, or whether there are no new ideas for instrumentation to address the needs of metallomics. In this presentation, answers to these questions will be considered. The answers to the first two questions are yes and yes; the final question will be addressed by offering examples of new devices that could be important for metallomics.
New mass spectrometric approaches are needed to address complex metallomic science problems. At EMSL, a DOE BER supported environmental user facility, we are developing a variety of new techniques that can help address the understanding of the role of metals in biological and environmental sciences. A new, ultra-high resolution 21T FTICR MS systems has been developed and commissioned at EMSL to provide users with the ultimate in mass spectrometric resolution and mass accuracy measurements. This system is also being used to develop a high spatial resolution imaging mass spectrometry system in combination with laser ablation electro spray ionization (LAESI) micro sampling approaches. Finally, new approaches using more conventional combined LC-MS and ICP MS techniques are being developed and applied to better understand the role and activity of metals in biological and terrestrial ecosystems. We will describe details and results of recent experiments using these new approaches.

Keywords: Atomic Spectroscopy, Environmental Analysis, Mass Spectrometry, Metals
Application Code: Environmental
Methodology Code: Mass Spectrometry
If nothing else, metallomics, in and of itself, is an incredibly broad topic. The combination of the number of potential biological systems/environments, the number of potential sample matrices, the number of potential metal “analytes”, and the range of potential analyte chemistries/masses/concentrations, is truly mind-boggling. The role atomic spectroscopy in metallomics has principally been in the use of ICP-MS as a highly-sensitive, element-specific detector, usually following some form of chromatographic separation. In this regimen, the ICP-MS is a standalone instrument, whose results must be integrated into parallel analyses usually involving separations and “organic” mass spectrometry. Unfortunately, these processes are not purely parallel in time, place, or methods of sample preparation/separation.

Over the last 5 years, this laboratory has developed the liquid sampling-atmospheric pressure glow discharge (LS-APGD) microplasma as an ionization source, initially for atomic mass spectrometry applications. Different from the ICP, the microplasma is directly coupled to mass spectrometers designed to sample electrospray ionization (ESI) sources; i.e. the vast majority of LC-MS instruments. In fact, the source has been coupled to single and triple quadrupoles, ion traps, and Orbitrap instruments. While not as sensitive as the ICP, isotope ratio performance on par with the state of the art sector instruments has been achieved on an Orbitrap platform. To complement the ability to perform elemental analysis, the microplasma can be operated using solvent systems commonly employed in LC-MS, producing “molecular” mass spectra that are essentially equivalent to those of APCI. Test compounds have ranged from small molecules (caffeine) to metalloproteins where the mass spectra are multi-charged intact molecules including metal constituents. We will present the use of the LS-APGD as a potentially highly-versatile source for metallomics analysis.
Metallomics: Does Analytical Science Still Have a Role?

Analytical Chemistry and Metallomics: An Unbreakable Association

The metallome has been defined as the entirety of metal and metalloid species (excluding non-metals) present in a cell or tissue type in terms of their identity, quantity and localization. Metallomic information can be attempted with different degrees of approximation, e.g. as a set of total element concentrations on the bulk level or at a specific location (e.g. a particular tissue or cellular compartment) or a set of metal complexes with a given class of ligands, e.g. metalloproteome (the entirety of metalloproteins) or metallometabolome (the entirety of metallometabolites). Regarding the development of specific analytical methods to cover all these aspects, the use of integrated mass spectrometric tools represents an attractive alternative, particularly in the case of dealing with the identification and quantification of metal complexes either in the case of metalloproteins or metallometabolites. However, new analytical methods are constantly demanded in order to address the presence of new metal species (e.g. nanoparticles) in biological systems.

In addition, the analysis of bulk concentration levels of metals and metalloids in a specific biological or environmental compartment has been satisfactorily approached by total elemental mass spectrometry (e.g. ICP-MS). However, it has been long known that biological/environmental populations are heterogeneous and that is more interesting to obtain data about individuals rather than settling for average information. Thus, mass spectrometric alternatives aimed to obtain information at the single cell level are nowadays gaining attention, particularly in combination with “fast scanning” mass analysers (e.g. TOF) to permit multi-elemental individual cell characterization.

These are some of the remaining challenges that analytical chemistry has to address to serve metallomics in the next years and that will be covered in the lecture.

Keywords: Bioanalytical, Clinical/Toxicology, ICP-MS, Nanotechnology

Application Code: Bioanalytical

Methodology Code: Mass Spectrometry
In this presentation, a new type of mass analyzer known as the distance-of-flight mass spectrometer (DOFMS) will be introduced. The DOFMS concept is best explained by comparison with traditional time-of-flight mass spectrometry (TOFMS). TOFMS determines the mass-to-charge (m/z) of an ion by imparting the same energy to all ions and then measuring the time required for each m/z to traverse a known distance and arrive at a detector. In contrast, DOFMS imparts the same momentum to all ions and then measures the m/z of an ion by determining the distance the ion travels during a set time period. Essentially, ions of lower m/z travel longer distances than ions of greater m/z, and their m/z can be calculated based upon their measured location. As a consequence of this strategy, the DOFMS can employ position-sensitive and array-based ion detectors to great advantage. Unlike TOFMS, which employs a single detector to detect all ions, a spatially-dispersive technique like DOFMS can employ an array of ion detectors, each of which can be tuned to best quantitate the ion flux at a given location (m/z). Here, both an electro-optic position sensitive ion detector and a new 1704-channel solid-state ion detector array will be examined for use within this role. Although DOFMS and TOFMS operate based upon very different concepts, both DOFMS and TOFMS can be made to employ the same instrumental architecture. Thus, both techniques are combined here into a single instrument to realize the advantages of both methodologies within a single instrument.
Microfluidic devices offer the possibility for point-of-care analysis of pathogens in remote settings, provided the devices are portable, require only minimal external instrumentation and little power and are robust. We have investigated a simple magnetic particle based extraction method, IFAST (immiscible filtration based on surface tension) for pathogen isolation from clinical and environmental matrices. Furthermore, we have experimented with paper microfluidic devices for colourimetric analysis of pathogen presence in veterinary samples.

Keywords: Bioanalytical, Chemiluminescence, Environmental/Biological Samples, Lab-on-a-Chip/Microfluidics
Application Code: Bioanalytical
Methodology Code: Microfluidics/Lab-on-a-Chip
Dengue virus (DENV) is an arthropod-borne virus transmitted primarily by Aedes mosquitoes and is major cause of disease in tropical and subtropical regions. Since the 1970's, it has spread to urban and semi-urban areas in over 100 countries and the incidence of dengue infections throughout the world has increased 30-fold. More than 3.6 billion people are at risk and an estimated 390 million infections occur annually. With no vaccine or specific treatment, early detection plays a significant role in decreasing fatality rates. Dengue infection has no pathognomonic clinical features, thus diagnostic tools are essential for diagnosis.

We report two nanobiosensors based on semiconducting single-walled carbon nanotubes (sSWNTs) chemiresistor functionalized with bioreceptors for the rapid, facile, low cost, point-of-care/use detection of DENV. The first biosensor detects whole DENV using Heparin, an analog of the heparan sulfate proteoglycans that are receptors for DENV, as a bioreceptor for detection of whole DENV virions. This permits detection of DENV virions from a variety of viral culture-compatible samples; such as fluid or tissue samples from monkeys, vector mosquitoes, and humans. In the second biosensor anti-dengue non-structural protein (NS1) monoclonal antibodies were used as a bioreceptor to detect DENV NS1, a clinically accepted biomarker for DENV infection, in Aedes mosquitoes and human saliva for early detection and diagnosis of the disease. The biosensors were selective and sensitive for their target analyte over the clinically relevant concentration range with detection occurring in only 10-20 min.

Keywords: Biosensors, Detection, Medical
Application Code: Biomedical
Methodology Code: Sensors
My research focuses on the use of computation/algorithms to create new optical microscopy, sensing, and diagnostic techniques, significantly improving existing tools for probing micro- and nano-objects while also simplifying the designs of these analysis tools. In this presentation, I will introduce a new set of computational microscopes which use lens-free on-chip imaging to replace traditional lenses with holographic reconstruction algorithms. Basically, 3D images of specimens are reconstructed from their “shadows” providing considerably improved field-of-view (FOV) and depth-of-field, thus enabling large sample volumes to be rapidly imaged, even at nanoscale. These new computational microscopes routinely generate >1–2 billion pixels (giga-pixels), where even single viruses can be detected with a FOV that is >100 fold wider than other techniques. At the heart of this leapfrog performance lie self-assembled liquid nano-lenses that are computationally imaged on a chip. The field-of-view of these computational microscopes is equal to the active-area of the sensor-array, easily reaching e.g., >20 mm^2 or >10 cm^2 by employing state-of-the-art CMOS or CCD imaging chips, respectively.

In addition to this remarkable increase in throughput, another major benefit of this technology is that it lends itself to field-portable and cost-effective designs which easily integrate with smartphones to conduct giga-pixel tele-pathology and microscopy even in resource-poor and remote settings where traditional techniques are difficult to implement and sustain, thus opening the door to various telemedicine and point-of-care related applications in global health. Some other examples of these smartphone-based biomedical tools that I will describe include imaging flow cytometers, immunochromatographic diagnostic test readers, bacteria/pathogen sensors, blood analyzers for complete blood count, and allergen detectors.

**Keywords:** Biomedical, Biosensors, Data Analysis, Microscopy

**Application Code:** Biomedical

**Methodology Code:** Portable Instruments
Point-of-Care Sensors for Emerging Infectious Diseases

Detecting Infectious Diseases Using Paper-based Analytical Devices

There is a continuing interest in the development of low-cost sensor systems to detect infectious diseases. Paper-based analytic devices have been used for centuries but a renewed interest in the substrate as a material for microfluidics started a decade ago when patterned paper was used to carry out multiplexed chemical analysis of urine samples. Since that time, the field has exploded in methods for fabrication, methods for detection, and applications. Our group has used paper-based devices for both environmental and clinical diagnostics with an emphasis on building devices that can be used in the field. To this end, recent results focused on detecting bacteria, including anti-microbial resistant bacteria, and viruses will be presented. We have shown that colorimetric chemistry can be developed with a range of selectivity from very specific (immunoassay and DNA-based) to general (enzyme-based). Use of the system to detect antimicrobial resistance in surface water will also be presented as an effort towards understanding horizontal transfer of resistance within the environment. While colorimetric detection can provide simple, instrument free detection, electrochemical detection is more sensitive and can provide more selectivity. We have developed two electrochemical paper-based devices for detection of bacteria and viruses. The first device incorporates thin wires functionalized with antibodies to detect a range of particle that mimic both viruses and bacteria. Testing with West Nile virus shows detection limits of approximately 1000 particles per mL. The second system incorporates carbon electrodes modified with pyrene. Using antibodies, detection limits of less than 20 particles can be achieved.

Keywords: Biosensors, Electrochemistry, Lab-on-a-Chip/Microfluidics
Application Code: Bioanalytical
Methodology Code: Microfluidics/Lab-on-a-Chip
# Point-of-Care Diagnostics for Infectious Diseases: Paper Microfluidics, Immunoassays, and Nucleic Acid Amplification Tests

Point-of-care diagnostics typically require devices that can carry out biochemical tests automatically, and ideally do so in a low-cost format without an instrument. The pregnancy test is the classic example of a disposable, instrument-free immunoassay for identification of a biomarker. Yet its simplicity comes at the cost of lower performance compared to lab tests and inability to carry out many of the powerful tests used in laboratory settings. At the University of Washington, we have exploited the wicking action of shaped paper to program multi-step timed assay events into simple passive devices. I will present our work in development of “paper microfluidics” and application to immunoassays and nucleic acid amplification tests.

**Keywords:** Biological Samples, Biosensors, Lab-on-a-Chip/Microfluidics, Nucleic Acids

**Application Code:** Bioanalytical

**Methodology Code:** Microfluidics/Lab-on-a-Chip
Until very recently, the conventional optical resolution limit for far-field infrared imaging was ~5-10 [micro]m, given the optics and the 2-25 [micro]m wavelengths of mid-IR. In 2011, the diffraction limit for far-field IR, ~1 [micro]m, was achieved with synchrotron source light and high NA optics illuminating a Focal Plane Array. Comparable capability for thermal source IR microscopes equipped with high magnification optics is now commercially available. Despite these advances, all microscopic imaging has been subject to the fundamental physical limitations of diffraction, viz., the inescapable fact that the size and separation of resolvable features is inversely proportional to the wavelength of the probing light, and directly proportional to the capability of the objective. The transformative breakthrough for infrared microscopy, Near-Field InfraRed (NFIR), is based on coupling the nanoscale capability of Atomic Force Microscopy with infrared spectroscopy and imaging. NFIR methods embody a conceptual paradigm shift, preserving the analytical power of IR spectroscopy, while breaking the infrared diffraction limit constraints for a thousand-fold increase in spatial resolution. We can now examine the chemistry of materials from micro to nano length scales, leading to a better macroscopic perspective.

I will present examples from our on-going research, including far-field imaging of cardiac tissue in a rat model of cardiac fibrosis and of biomass in Arctic sea ice diatoms, as well as NFIR imaging of nuclear pores in cell nuclei, comparable to super-resolution fluorescence imaging of similar targets. Molecular interactions that occur below the diffraction limit have consequences on length scales from molecular to macroscopic. In the future, these spectroscopic and imaging capabilities will facilitate and accelerate new avenues of investigation in materials, environmental and health research.

Keywords: Imaging, Infrared and Raman, Microscopy, Nanotechnology
Application Code: Bioanalytical
Methodology Code: Vibrational Spectroscopy
Generally, neurotransmitter concentration is probed in vivo by drilling holes in the skull to access the brain. Our group focuses on the detection of biomarkers for neurological activity in non-invasively collected biofluids and through the skull. Our approach is to apply surface enhanced Raman spectroscopy (SERS), which provides greatly enhanced Raman signals from very low concentration analytes that have been adsorbed to metal nanoparticles, for the detection of neurotransmitters. The metal nanoparticles create an oscillating electric field called the localized surface plasmon resonance (LSPR) when excited with a laser, which results in the enhancement of the weak Raman signal. SERS is surface selective, highly sensitive, rapid, label-free and requires little to no sample processing. Raman scattering spectra can be obtained from subsurface layers of turbid media through collection of Raman scattered light at a location that is spatially offset from the incident illumination point. This technique, known as spatially offset Raman spectroscopy (SORS), is combined with SERS to develop the technique termed as surface enhanced spatially offset Raman spectroscopy (SESORS). We present results on the SERS detection of neurotransmitters in the micromolar ([micro]M) to nanomolar (nM) concentration range in aqueous solution and SESORS measurements of neurotransmitters through a skull.

Keywords: Neurochemistry, Raman Spectroscopy, Surface Enhanced Raman Spectroscopy
Application Code: Neurochemistry
Methodology Code: Vibrational Spectroscopy
It has long been known that some guanosine nucleosides and nucleotides can self-assemble in aqueous solution to form reversible gels under certain experimental conditions. The building blocks of the guanosine gels (G-gels) are guanine tetrads formed through hydrogen bonding between each guanine and its two neighboring guanines. These tetrads can then self-assemble into helical stacks of tetrads through pi-pi interactions. As concentration of the monomeric guanosine compound increases, further self-assembly can occur to form highly ordered cholesteric and hexagonal liquid crystalline gel phases. G-gels formed by individual guanosine compounds exhibit thermodissociative behavior, forming gels at low temperature that melt when temperature is raised above a certain temperature. Several years ago we discovered that G-gels formed by mixtures of the nucleotide 5'-guanosine monophosphate (GMP) and the nucleotide guanosine (Guo) exhibit novel thermoresponsiveness: in some compositions, the gels are thermodissociative as expected, i.e., they gel with decreasing temperature; in other compositions, however, the gels are thermoassociative - they begin as liquids at low temperature, forming gels above some critical temperature, and melt above a second critical temperature. The temperature range of gelation is highly tunable as a function of total GMP+Guo concentration, GMP/Guo ratio, cation content, pH, and ionic strength. Due to their helicity, G-gels are chiral and can be studied using circular dichroism spectroscopy. This talk will describe circular dichroism investigations of the liquid-gel transitions in the GMP-Guo solutions, to support applications of these biocompatible gels in areas such as protein and cell encapsulation, nanoparticle solubilization, and chiral separations.

Keywords: Characterization, Chiral, Material Science, UV-VIS Absorbance/Luminescence
Application Code: Material Science
Methodology Code: Molecular Spectroscopy
Surface enhanced Raman scattering (SERS) is an analytical technique with several advantages over competitive techniques in terms of improved sensitivity and multiplexing. We have made great progress in the development of SERS 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 SERS 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 functionalised nanoparticles as sensing materials for the detection of bacteria and proteins at ultra low levels. We achieve this by combining functionalised silver or gold nanoparticles with SERS. This combination can result in extremely sensitive and selective biosensing with unprecedented multiplexing capabilities.

Keywords: Bioanalytical, Biosensors, Raman Spectroscopy, Surface Enhanced Raman Spectroscopy
Application Code: Bioanalytical
Methodology Code: Vibrational Spectroscopy
The last decade has seen many advances in spectroscopic techniques for characterization of complex pharmaceutical molecules such as chiral organics and biologics. Among the most advanced, new and innovative techniques have been vibrational spectroscopy based Vibrational Circular Dichroism (VCD), Raman Optical Activity (ROA) and rotational spectroscopy known as Molecular Rotational Resonance (MRR). These techniques were all developed and commercialized in order to bring simplicity and speed to analysis. For example, regulatory agencies require knowledge of absolute configuration, enantiomer purity and diastereomeric ratio or impurity for an NDA approval of a chiral entity. The ‘traditional’ analysis involved x-ray crystallography and depended on the molecule’s ‘ability’ to crystallize. VCD, on the other hand, solves the stereochemistry in solution without the need to grow a crystal; it is easier, faster and less expensive. Diastereomeric purity has always been measured via separation techniques that involve not only sophisticated method development but significant expense and time. MRR can precisely resolve diastereomers in a complex mixture and do so on-line – without a need for offline sample preparation. And ROA has been recently shown to exhibit an increased sensitivity to Higher Order Structure of biologics over the standard techniques of CD, FT-IR and Raman. In this presentation, we will discuss how these groundbreaking spectroscopic techniques are changing the landscape of analytical chemistry in pharma.

Keywords: Biopharmaceutical, Chiral, Pharmaceutical, Vibrational Spectroscopy
Application Code: Pharmaceutical
Methodology Code: Vibrational Spectroscopy
Biomedical institutions, such as medical schools and centers, offer bioengineering researchers valuable resources to potentially augment the impact of engineering discoveries. Biosensor development benefits from collaborations that give in-depth knowledge of the biological systems under assessment and existing bioassay technologies, as well as access to biomedical core facilities and biological samples. Biomedical institutions, however, do not always offer the same types of access to students and service opportunities as most academic research institutions, requiring innovation in shaping broader impacts-related activities, for instance, for NSF proposal development. NSF CAREER biosensor proposal development from a non-traditional bioengineering setting will be discussed.

Keywords: Bioanalytical, Biomedical, Biosensors
Application Code: Bioanalytical
Methodology Code: Sensors
Research funding, as is true of many opportunities, undergoes shifts. As science advances, funding levels change, and priorities are altered. Researchers should employ savvy strategies when seeking support. Innovative research ideas, coupled with well-composed proposals aimed at identified areas of interest to funding organizations, are among the important factors for successful applications. Funding agencies around the world are supporting the development of emerging technologies, including nanotechnology. A critical factor for successfully implementing and managing nanotechnology, realizing the benefits while minimizing detriments, requires examination of the interactions of these particles at interfaces within biological and ecological systems. Previous research exploring toxicity, fate and transport, and exposure assessments for nanoparticles has increased more than tenfold in the decade between 1995 and 2015. The resulting information has provided data snapshots of selected nanoparticles in specific media, at selected time points of the material/particle life cycle. It is clear that a more strategic research focus that probes the basic mechanistic behavior of nanoparticles is critical. Research opportunities within the Chemical, Bioengineering, Environmental and Transport Systems (CBET) Division's Nano Program within the Engineering Directorate of the National Science Foundation (NSF) are provided in this presentation. In addition, information about the National Nanotechnology Initiative will also be discussed.

Keywords: Environmental Analysis, Environmental/Waste/Sludge
Application Code: Environmental
Methodology Code: Education/Teaching
National Science Foundation (NSF) - Funding Opportunities for Nano-Biosensing and Early Career Inves

The Chemical Measurement and Imaging program at the National Science Foundation is a source of support for much of the fundamental research relevant to chemical measurement science. The program description touches on research related to atomic and molecular spectroscopy, chemometrics, electroanalytical chemistry, chemical imaging, magnetic resonance, mass spectrometry, sensors, and separations (inter alia). There is also an increasing interest with the CHE division for data-driven discovery and mid-scale instrumentation development. This presentation will provide an overview of the CMI program, where and whether a research project may fit in this program and in the CHE division. It will also provide an opportunity to discuss new funding initiatives at NSF and within CHE.

Abstract Text

The Chemical Measurement and Imaging program at the National Science Foundation is a source of support for much of the fundamental research relevant to chemical measurement science. The program description touches on research related to atomic and molecular spectroscopy, chemometrics, electroanalytical chemistry, chemical imaging, magnetic resonance, mass spectrometry, sensors, and separations (inter alia). There is also an increasing interest with the CHE division for data-driven discovery and mid-scale instrumentation development. This presentation will provide an overview of the CMI program, where and whether a research project may fit in this program and in the CHE division. It will also provide an opportunity to discuss new funding initiatives at NSF and within CHE.

Keywords: Data Analysis, Imaging, Instrumentation, Method Development

Application Code: Other

Methodology Code: New Method
Research funding, as is true of many opportunities, undergoes shifts. As funding levels change, priorities are altered, and application numbers vary, applicants should employ savvy strategies when seeking support. The integration of nanotechnology and biosensing technologies has been attracted substantial research efforts due to the broad applications in healthcare, environmental, homeland securities and agricultures, and this is reflected in the continued growth of global markets for such technologies.

**Keywords:** Bioanalytical, Biomedical, Biosensors, Education

**Application Code:** Bioanalytical

**Methodology Code:** Sensors
In this presentation, I will present my perspective of developing and sustaining a research program in nano and biosensing based on my experience with my own program, with observations gained during my service to sensors conferences as organizer, as editor and reviewer for sensors journals, and as reviewer for federal agencies with particular emphasis on the National Science Foundation. I will first briefly present an outline of my overall research program that includes biosensors as a significant component and how the program is tied together at the fundamental level. I will then present how proposal projects emerge for federal agencies in sensing, and also in related areas from my program, with specific emphasis on NSF and NIH as targeted agencies for these proposals. I will then present my experience as a reviewer for NSF over a fairly long time, specifically on sensors panels, and summarize some principles I have learnt that could help the audience on elements that could be responsive to solicitations and attractive to panalists and panels, while allowing the researchers to sustain their research programs in this tight funding environment. While the emphasis is on NSF projects and funding, in keeping with the main aim of this talk of providing a perspective on sustaining a sensors research program, I will discuss also the role of innovation, and the role of application in sustaining interest in university research and a research program.

Keywords: Biomedical, Sensors
Application Code: Biomedical
Methodology Code: Sensors
My NSF CAREER award from the Nano-Biosensing is focused on the development of optical sensing and imaging technologies for detection of infection, particularly pulmonary tuberculosis. One third of the world’s population is infected with Mycobacterium tuberculosis; only 5-15% of infections develop into active tuberculosis. There is an urgent need for sensing technologies to study the mechanisms of pathogenesis and to aid in the development of effective vaccines and antibiotic therapeutic agents, particularly to combat multidrug-resistant tuberculosis. Using a fiber optic microendoscope for intravitral excitation of fluorescent bacteria in the mouse lung, we have improved the threshold of detection of whole-animal imaging by 2-3 orders of magnitude, using near infrared and visible wavelengths. We have also developed computational models of light transport in the lung and tissue phantoms to evaluate detection in larger animal models. The project integrates the technology research with educational programs through the global health focus of the project. A summer study abroad program in Rwanda provides training of undergraduate students in medical equipment and the challenges of design, operation, and maintenance of medical technology in the developing world. Students perform a needs assessment, define a design project, and work in multidisciplinary design teams back on campus at Texas A&M to create low-cost, appropriate technology solutions. These international experiences are transformational for the participating students and immensely beneficial to the overall research project and to a globally-engaged engineering workforce.

Keywords: Biomedical, Biosensors, Fiber Optics, Fluorescence
Application Code: Biomedical
Methodology Code: Fluorescence/Luminescence
The Latest Developments in (U)HPLC Column Technology

Optimization of Flow Paths Through the LC to the Electrode in New Ion Sources Drives Ease of Use

One of the long running critiques of micro and nano LC, with or without an MS, is the fiddley nature of setting up the instrument and getting useful and reproducible data. In the hands of an expert the techniques are incredibly powerful. In the hands of a novice they are frustrating. We have put together a pump to sprayer solution (LC, Column Oven, Columns, Connectors, Ion Source, and electrodes) enabling “no-adjustment” set up and spray for nano and micro LC/MS allowing these technologies, and the increased sensitivity they provide, to be used by a broader range of users. Data will be shown comparing to well-tuned sources in expert users hands, comparisons of set up time, and robustness of the systems.

Keywords: Bioanalytical, HPLC, HPLC Columns, Liquid Chromatography/Mass Spectroscopy
Application Code: Bioanalytical
Methodology Code: Liquid Chromatography/Mass Spectrometry
High-performance liquid chromatography (HPLC) columns, bearing alternative stationary phase chemistries, are commonly applied in small molecule applications. There has been considerable research conducted using small molecule probes to provide fundamental information regarding the molecular interactions that contribute to differing selectivity and retention. Reversed-phase separations of large molecules, however, tend to employ very few stationary phase chemistries. Can some of the advantages observed for alternative stationary phase chemistries, as applied to small molecule analyses, be observed in larger molecule applications?

Initially, peptide probes are designed to highlight molecular interactions such as hydrogen bonding, pi-pi and ion-exchange mechanisms. The probes are chromatographed using wide-pore stationary phases functionalized with common alternative ligands more commonly used for small molecule separations. Retention and selectivity of the probes using varied mobile phase conditions are then compared to the same base particles modified with alkyl modifications to elucidate important molecular interactions and identify conditions where alternative selectivity is highlighted. Finally, more traditional large molecule workflows are compared with these new approaches.

Keywords: Bioanalytical, HPLC Columns, Liquid Chromatography, Peptides
Application Code: Bioanalytical
Methodology Code: Liquid Chromatography
Hydrophilic interaction chromatography, or HILIC, is a multimodal separation technique where analytes separate by partitioning between a water-rich layer on a polar stationary phase and the mobile phase, which is typically a mixture of an aprotic solvent with small amounts of water. This separation technique is advantageous for mixtures of complex, polar analytes which would otherwise not retain on reversed phase HPLC columns. However, some HILIC applications can be particularly difficult. For example, monosaccharides and disaccharides typically have critical pairs like lactose and maltose which co-elute using standard HILIC columns and conditions. Another application, fluorophore labeled N-linked glycans, present other challenges, especially with negatively charged, complex glycans. Here, we look at two novel, polar stationary phases for these difficult HILIC separations. Additionally, we look at how core-shell morphology can improve chromatography as well as throughput.
Although liquid chromatography is already often considered as a mature technique, the innovations in columns and instrument technologies over the last decade have improved separation efficiency by a factor of around 4 (in the same time) or reduced analysis time by a factor 16 (for the same efficiency). Given the fact that separation quality (resolution) is proportional to the square root of efficiency, it is impossible to further improve the separation to such an extent that very complex samples, containing 1000 or more components, can be separated in a one dimensional setting. Nevertheless, the development of column technology remains of paramount importance as they remain the core of each dimension in multidimensional separations. Further improvements in column efficiency can however only be achieved if two key features are met: the reduction of extra-column dispersion that has become the dominant contributor to the overall performance and a solution for the deleterious effects of viscous heating that occur when operating columns at very high pressure.

Several routers are possible to further improve columns performance. Where the introduction of core-shell particles shifted the minimum achievable plate heights down by 30-40%, new technologies such as micelle templated radially oriented pores, microfabricated columns and the introduction of 3D printing techniques, although still in a development phase, show tremendous possibilities. This contribution will review the current limiting effects in one dimensional separation performance, in addition to illustrating current and future developments in column technology and instrument design.

Keywords: HPLC, Liquid Chromatography
Application Code: General Interest
Methodology Code: Liquid Chromatography
The Latest Developments in (U)HPLC Column Technology

**Abstract Title**
Development of Ultra-High Resolution Microfluidic Columns

**Primary Author**
Fabrice Gritti
Waters Corporation

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**Abstract Text**

Capillary (150-300 [µm] i.d. x 100 mm) columns are advantageous for the identification and quantification of compounds by LC-MS analyses with respect to conventional narrow-bore columns for four main reasons: 1) the applied flow rate (1-10 [µL/min]) is compatible with maximum ionization efficiency for MS detection, 2) the reduction of the column volume increases the limits of detection, 3) viscous heating is negligible at such small flow rates, and 4) capillary columns reduce solvent consumption, therefore, lower the analysis cost.

The achievement of a uniform packed bed in capillary columns remains highly challenging for sub-2 [µm] particles. Additionally, the presence of conventional frits (porosity 0.2 [µm]) and metallic endfittings cause severe band spreading. As a result, the resolution power of these capillaries columns remains lower than that expected.

Solutions to these problems are proposed in this presentation. A series of 250 [µm] x 100 mm prototype capillary columns and microfluidic channels (150 and 300 [µm] equivalent i.d.) were packed with 1.8 [µm] high strength silica (HSS)-C18 fully porous particles at 20 kpsi. High slurry concentrations are used. They are prepared free from the usual frits and metallic endfittings. First, their efficiency (isocratic runs) and peak capacity (gradient runs) are accurately measured using ultra low-dispersive nanoLC instruments. MicroHPLC performance is then compared to that of 2.1 mm i.d. columns packed with the same particles. Secondly, improvement of the column performance is achieved by combining the solvent gradient with traversing temperature gradients. Finally, LC-MS analyses of peptides using such microfluidic channels are presented.

**Keywords:**
Capillary LC, Liquid Chromatography, Peptides

**Application Code:**
General Interest

**Methodology Code:**
Liquid Chromatography
The Latest Developments in (U)HPLC Column Technology

New Surface Chemistry and Particle Design for HPLC Columns

In recent years superficially porous particles (SPPs) have drawn great interest because of their special characteristics and improvement in separation efficiency. SPPs are manufactured by adding silica nanoparticles onto solid cores either using a multi-layer or one-step coacervation process. The pore size is mainly controlled by the size of the silica nanoparticles and the tortuous pore channel geometry is determined by how those nanoparticles randomly aggregate. Currently SPPs are available with a pore size from 80 to 1000 Å and more than 10 chemistries for analyzing small molecules, peptides, proteins and monoclonal antibodies.

Here we report a new hybrid endcapped zwitterionic chemistry made on 120 Å pore size, 2.7 µm SPPs. The new chemistry can be used in hydrophilic interaction chromatography (HILIC) with high-pH stability for carbohydrates, pesticides and metabolomics analysis. Another development is on a new particle design by forming unique, elongated pore channels normal to the surface. Reduced plate heights as low as 1.0 on columns packed with the new SPPs have been observed. The improvement in separation efficiency can be attributed to a significant decrease in the B-term band broadening because analytes can only diffuse anisotropically within these straight, unconnected, radially oriented pore channels.

Keywords: Chromatography, HPLC, HPLC Columns, Liquid Chromatography

Application Code: General Interest

Methodology Code: Liquid Chromatography/Mass Spectrometry
Recently our group developed a colorimetric/fluorimetric detection method of residual metal in the pharmaceutical compounds such as Active Pharmaceutical Ingredient (API), its process intermediates and the final drug product. As we expand this approach toward base metal ions such as copper, zinc, iron and nickel, a series of colorimetric/fluorimetric copper probe molecules have been investigated. Such molecular probes share a common scaffold, ortho-(phenylazo)aniline, which undergoes oxidative cyclization in the presence of copper (CuII). The reactivity toward copper ions is studied by systematically adjusting the electron-donating groups (EDG) in the molecular probes. Not only the number of EDGs but also the position of EDGs shows markedly different kinetic rates upon exposure to copper ions. Among the candidates, the fastest (40-times faster than benchmark) and most sensitive (20-times lower detection limit than benchmark) probe molecule was identified. With this probe molecule in hand real-world samples from Merck were tested to further develop a user-friendly copper screening method. In the presentation, the progress of the development and structure-reactivity relationship will be discussed.

Keywords: Fluorescence, Pharmaceutical, Sensors

Application Code: Pharmaceutical

Methodology Code: Sensors
Elemental Analysis in Pharmaceutical Research, Beyond Elemental Impurities

Chemical Imaging Based on Laser Ablation-Based Techniques

Laser Induced Breakdown Spectroscopy (LIBS) and Laser Ablation Inductively Coupled Plasma-Mass Spectrometry (LA-ICP-MS and ICP-OES) are used simultaneously for spatially resolved mapping of major and trace elements and isotopes from a wide variety of solid samples. The combination of the two techniques provides complementary measurements for elements that are separately unattainable due to low sensitivity and/or strong interferences. Two dimensional (2D) layer-by-layer mapping, 2D cross-sectional imaging and three-dimensional (3D) volume rendering of elements and isotopes in these samples are presented.

Keywords: Analysis, Bioanalytical, Pharmaceutical, Surface Analysis
Application Code: Pharmaceutical
Methodology Code: Atomic Spectroscopy/Elemental Analysis
Measurement of residual metals in pharmaceutical intermediates is routinely performed using inductively coupled plasma-optical emission spectroscopy (ICP-OES) or inductively coupled plasma-mass spectrometry (ICP-MS). However, these techniques suffer from drawbacks that include slow turnaround. We investigate the use of a fast and inexpensive high-throughput approach for quantification of residual palladium (Pd), based on the Pd-catalyzed Tsuji-Trost deallylation of an allylic ether substrate to produce a highly fluorescent product. We demonstrate the effectiveness of this fluorescence assay for accurate quantitation of Pd levels in a variety of samples produced at Merck. We also developed a fluorometric method for quantifying trace copper in complex samples. Both methods are based on catalysis, providing sensitivity that match that of ICP-MS.

Keywords: High Throughput Chemical Analysis, Metals, Sensors, Trace Analysis
Application Code: High-Throughput Chemical Analysis
Methodology Code: Fluorescence/Luminescence
To address new and demanding USP <232> and ICH Q3D elemental impurity requirements, a significant investment in state-of-the-art instrumentation was necessary to overcome challenges in achieving robust, ultra-trace, quantitative measurements for an array of drug products. Single-reaction-chamber microwave systems offer the flexibility and robustness needed to prepare diverse sample matrices, from highly organic materials to difficult-to-digest silicates. Ultra-trace multielemental analysis is enabled by the sensitivity and unparalleled selectively provided by ICP-QqQ-MS/MS instruments, which can overcome the most severe isobaric interferences. For investigating elemental impurities, this combination of high-end capabilities has become our “platinum standard” for greatly reducing the method development cycle time and providing the highest assurance of accurate results, with modest effort.

Our investment in new capabilities has opened the door to create technical insights, well-beyond addressing elemental impurity compliance needs. These techniques have enabled product quality assessments, raw material characterization, clinical study measures, mechanistic understanding, and creation of models to predict or assess product performance. Analytical challenges are similar to those faced in elemental impurity compliance work, so many strategies developed for ultra-trace analysis can be reapplied. Additionally, these state-of-the-art capabilities have expanded the portfolio of elemental analysis applications to include trace measurement of less-traditional elements, such as S, Br, and Cl.

This presentation will highlight representative analytical challenges and strategies that have created new opportunities to change the game within our health care and consumer goods R&D environment.
Laser Induced Breakdown Spectroscopy (LIBS) has emerged as an innovative tool for quantitative and qualitative elemental analysis in pharmaceutical research. The sampling process, laser ablation (LA), involves a high-power pulsed laser beam directed and focused onto a solid sample to convert a finite volume of the sample into excited vapor and aerosol constituents. As the plasma cools, the excited constituents relax to their ground state and emit light at characteristic wavelengths of the elements present. LIBS has several advantages including little to no sample preparation, fast measurement time, broad elemental coverage, and versatile sampling protocols. Samples for LIBS experiments can be any form: solid, liquid or gas. Importantly, LIBS can detect both organic and inorganic elements of the sample simultaneously, which is impossible in common techniques such as ICP-OES or ICP-MS. With these attributes combined LIBS has become of significant interest in pharmaceutical research. Herein, the potential use of LIBS for rapid characterization of tablet coatings will be illustrated, including the investigation of the both intra- and inter-tablet variability. Firstly, the thickness and uniformity of the tablet coating, which has a significant effect on the coating performance, was assessed by LIBS. Additionally, the technique was used to locate contaminants on the coating, as well as to identify the specific elemental contaminants present. Further, principle component analysis (PCA), which can effectively reduce the high-dimensional LIBS data onto a lower dimensional coordinate system and thus discriminate among different samples was utilized to visualize differences in tablet coatings. To date, there are only a few examples using LIBS in the pharmaceutical industry, and no literature on thorough investigations of both intra- and inter-tablet coating variability by LIBS.

Keywords: Materials Characterization
Application Code: Pharmaceutical
Methodology Code: Atomic Spectroscopy/Elemental Analysis
The presence of residual elemental impurities is an issue of increasing importance and concern to regulatory agencies around the world to ensure patient’s safety. Several common metals such as cadmium, lead, mercury, and arsenic are known to negatively impact human health and agencies have set strict exposure limits to minimize their exposure. In addition, toxicological data has also led the regulatory agencies to limit the exposure levels of other elemental impurities used in API manufacturing. Metal catalyzed reactions are standard practice in modern day API manufacturing industry. Metals are extensively used in cross-coupling reactions including Pd catalyzed Suzuki reactions and Cu catalyzed azide-alkyne cyclo additions to enable c-c bond formation under mild conditions. Elemental impurities could originate from multiple sources including catalysts, raw materials, glassware, reactors; equipment used in storage and handling of starting materials, intermediates, reagents at different stages of API synthesis. The detection of residual metallic impurities in APIs has evolved considerably in the past century since USP <231> was first issued 100 years ago. The USP colorimetric method for heavy metals is sensitive for few metals of concern, however, lacks sensitivity and specificity for other metals of toxicology concerns like mercury and cadmium. Instrumental methods such as ICP-OES and ICP-MS are highly sensitive and can perform multi-elemental analysis. To determine the residual elemental impurities levels present in API samples, a generic ICP-MS method was successfully developed and validated for twenty two elements specified in ICH Q3D (Li, V, Cr, Co, Ni, Cu, As, Mo, Ru, Rh, Pd, Ag, Cd, Sn, Sb, Ba, Ir, Pt, Au, Tl, Pb and Hg) commonly used in API synthesis of small molecules compounds. This presentation will highlight the universal method development and importance of ICP-MS for quantitative analysis of elemental impurities in pharmaceutical Industry.
Spectroscopy for the analysis of foods has a long history. The analyses are conducted in the laboratory with skilled analytical chemists using laboratory based instrumentation. Over the past XX years there has been a dramatic decrease in the size and an increase in the capabilities of miniature spectroscopic systems. The enabling technologies have changed dramatically which has aided moving the analysis from the lab into the field. This revolution was led by miniaturization of photonics devices, major improvements in data processing capabilities and examples of small hand-held instruments for first responders.

But being ABLE to make a measurement is not the only requirement. Food samples are by their nature heterogeneous. So what makes a representative sample? Food samples contain water. How does the amount of moisture in the sample impact the results? In a laboratory environment these factors would be investigated and controlled. But because of the revolution in the ability to acquire and process data, many companies are trying to place these devices in the hands of the consumers. They are also advocating crowd-sourced data that introduces another set of errors.

This paper will discuss the pitfalls and “gotchas” that are not being addressed in light of these new developments.

Keywords: Data Analysis, Food Identification, Molecular Spectroscopy, Sampling
Application Code: Food Safety
Methodology Code: Molecular Spectroscopy
The methodology behind developing a robust application for material identification or quantitative analysis is often poorly understood or poorly implemented. A properly designed chemometric approach to data analysis is required in order to develop models that can be consistently applied and will stand the test of time. Along with properly designed sampling approaches, an analytical approach to model development and the correct use of chemometric tools is essential to both identification, and especially for quantitative analyses.

Examples will be provided to illustrate some of the approaches required to develop robust models for both identification and also quantitative analysis. Examples will focus on NIR quantitative applications in food/agricultural and also identification and library development in portable spectroscopic instruments.

**Keywords:** Chemometrics, Method Development, Spectroscopy, Statistical Data Analysis

**Application Code:** Food Identification

**Methodology Code:** Chemometrics
In recent years the adulteration and mislabeling of high value foods has become more prevalent. One example of these commonly targeted foods is herbs and spices, especially those that command a higher price such as saffron. Herbs and spice fraud can be carried out by different means from sophisticated dilutions of cinnamon bark oil with cinnamon leaf oil to adding talc to powders to add weight. To detect these types of adulteration various lab techniques can used, usually based on chromatography with mass spectrometry. These techniques are very accurate and are definitive at identifying fraud however they are time consuming and as such are invariably carried out on statistical fraction of all received spice samples furthermore they are targeted techniques so you maybe analyzing for one threat and missing another. This is where screening techniques such as FT-IR and FT-NIR have an advantage being quick and having no sample preparation so can be carried out on all foods being received. They are also normally smaller and more robust than other lab technologies so can be utilized in a food processing environment. Furthermore they can be used in a non-targeted fashion, identifying any possible threats rather than one specific concern. For this work therefore, we chose FT-NIR to investigate its ability to identify adulteration in a wide range of herbs and spices.
Food, Photonics and Field Spectroscopy – Are All the Components Available and Understood?

The “Star Trek” Tricoder is Becoming a Reality for the Food Industry – Application of Portable and Handheld Sensors for Monitoring Quality in Food Products

Optical technology is rapidly developing and instruments are available commercially as portable, hand-held, and micro-devices that can be used when it is not practical or economical to use the more sophisticated and costly instruments used in research laboratories. We will present information on the feasibility of handheld and portable vibrational spectroscopy systems in applications relevant to the food industry. We have evaluated the performance against benchtop systems directed at developing fingerprinting strategies for rapid and specific analysis of high-risk foods and contaminants, providing reliable tools for assessment of adulteration and safety. Food applications have been targeted on detection of chemical food contaminants through development of spectral signature profiles permitting the chemically authentication of raw materials. This technology can enable the food manufacturer for real-time and field-based measurements to control the raw material stream, addressing safety and brand equity. Portable infrared technology could save time and money to the food industry helping to implement risk management systems.

Keywords: Food Contaminants, Food Identification, Portable Instruments, Quality Control
Application Code: Food Science
Methodology Code: Vibrational Spectroscopy
Session Title: Food, Photonics and Field Spectroscopy – Are All the Components Available and Understood?

Abstract Title: On-Site Food Safety Screening with Raman

Primary Author: Katherine A. Bakeev
Author: B&W Tek

Co-Author(s): Chen Qizhen, Chris Ye, Jack Zhou, Philip Zhou

Abstract Text
Food safety testing is becoming increasingly important as global supply and demand of food changes. There have been many instances of economically-motivated food contamination that have led to serious incidents including death. There is a need for on-site testing methods that can be done rapidly and accurately at a cost that can keep food safe and affordable. Perishable foods, such as produce must be tested while they are still fresh. The testing must not only be rapid, but preferably be able to be done throughout the life of the product from farm to table. The testing must also have the sensitivity to detect a range of compounds including pesticide residues, food additives, residual veterinary medicines as well as other contaminants. We will discuss field screening Surface-enhanced Raman spectroscopy (SERS) method with specialized reagents for different compound classes. This rapid screening tool that can be used for food testing by producers, food processors, food inspectors, and food suppliers (supermarkets) to screen for potential issues in foods from fresh produce, to processed products and nutritional supplements to give an assurance of food safety.

Keywords: Food Safety, Pesticides, Raman Spectroscopy, Surface Enhanced Raman Spectroscopy
Application Code: Food Safety
Methodology Code: Portable Instruments
The performances of handheld devices and/or benchtop mid-infrared (IR), near-IR (NIR) and Raman spectrometers were evaluated for the rapid screening of extra virgin olive oil (EVOO) for authenticity. The univariate conformity index and the multivariate supervised soft independent modeling of class analogy (SIMCA) classification tools were used to differentiate among the various oils investigated. A novel FT-NIR and partial least squares (PLS) procedure was also applied to EVOO to rapidly predict whether the oils are authentic, potentially mixed with refined olive oil (RO) or other vegetable oil(s), or of lower quality. This methodology involved estimating the newly coined FT-NIR Index, predicting the concentration of five fatty acid (FA) markers, and predicting the concentration of RO and the nature and concentration of other edible oils using gravimetrically prepared mixtures with EVOO. These mixtures were EVOO spiked with edible oils high in linoleic acid (OH-LNA), oils high in oleic acid (OH-OLA), palm olein (PO), and RO. The FT-NIR Index provided an estimate of total volatiles characterized by an overtone band attributed to a carbonyl group absorbing near 5269 cm⁻¹. Based on the analyses of 88 commercial products labeled EVOO with various instruments, 38-44% were classified as belonging to the class of authentic EVOO or satisfied the requirements identified for predicting authentic EVOO products.

Keywords: Chemometrics, Food Safety, Lipids, Vibrational Spectroscopy
Application Code: Food Science
Methodology Code: Vibrational Spectroscopy
The notion of a 'lab' is taking a new meaning thanks to new sensors, increased computing power of smartphones and cloud computing. On one hand, today's smartphones boast of 6GB RAMs, 2GHz processors and a range of wireless communication approaches with the availability of programmable open source components. On the other hand, miniaturized spectrometers have been realized due to MEMS based fabrication approaches. The merger of these technologies offers promising opportunities for a range of applications that were not feasible earlier. 'Millennial spectrometers' are sleek, miniature, portable and seamlessly integrate into smartphones via apps and can be made more effective through cloud computing. It will be possible to generate large spectroscopic datasets for a range of food, beverage and agricultural products that will pave the way for intelligent sensors that 'learn' over time and enable new applications like counterfeit detection, predict harvest times, improve distribution of produce among other uses. It shouldn't be long before we will truly have a lab-on-a-phone!

Keywords: Consumer Products, Food Science, Portable Instruments, Spectrometer
Application Code: Food Science
Methodology Code: Portable Instruments
Hyperspectral imaging is a combination of spectroscopy and digital imaging, producing data products where each pixel in an image has a high resolution optical spectrum. Techniques for image processing can be used to identify regions of interest in captured imagery, and techniques for spectroscopic analysis can be applied to the pixels in selected regions to identify materials. Hyperspectral systems can be flown on aircraft for analysis of crops and forests, used in the lab for detailed analysis of materials or products, and mounted over conveyor systems for online analysis of food products during processing.

In agriculture, hyperspectral imagery can be used to differentiate species and cultivars of plants in greenhouses or in the field. In addition, many measures of crop health and stress can be derived from spectral analysis of hyperspectral imagery. Agricultural products can be analyzed for quality, contamination and the presence of foreign material.

This paper will discuss the general process of generation and collection of hyperspectral imagery, and then illustrate several examples of how these systems have been used in automated analysis of plants and agricultural products. Example applications include automated sorting of almonds using robots in a factory setting, detection of contamination in produce and poultry and analysis of the composition of processed food products. Hyperspectral imaging has a broad range of demonstrated and potential applications to increase food quality and reduce costs in large scale farming and food processing.

Keywords: Food Safety, Food Science, Identification, Imaging
Application Code: Food Safety
Methodology Code: UV/VIS
Recently, the legal landscape for cannabis has changed considerably within the United States and for many other countries. The US cannabis industry needs a wide range of chromatographic methodologies to fit within the new landscape. Analytical testing for the different cannabinoids is often the first chromatographic methodology that is developed by company that is new to the industry and this testing is typically a liquid chromatography application. Terpenes are another class of compounds that are of interest to the cannabis industry. Analytical testing for terpenes is often accomplished with gas chromatography. Pesticide analysis is currently (or soon will be) legally required for commercial cannabis products. Pesticide testing is often done by gas or liquid chromatography with mass spectrometry. Preparative liquid chromatography has a role in the cannabis industry for isolating individual components and for concentrating similar compounds. The unique properties of SFC make it a suitable platform for these chromatographic methodologies.

The work presented here will demonstrate SFC-UV and SFC-MS applications for the cannabis industry. Examples of analytical methodology for purity / potency and identification of cannabinoids will presented. SFC applicability for analytical methodology of terpene and pesticide analysis will be discussed and examples will be presented.

Keywords: Mass Spectrometry, Method Development, Pesticides, SFC
Application Code: Food Science
Methodology Code: Supercritical Fluid Chromatography
Carmine is widely used in food industry as a colorant. The coloring principles in carmine consist mainly of carminic acid. The existing carmine standard in Food Chemical Codex (FCC) assays carminic acid with spectrophotometry by measuring absorbance. However, the spectrophotometry may overestimate the carminic acid due to some potential interfering substances in carmine. Adulteration was reported for carmine. There is a need to modernize the FCC carmine standard by replacing the existing spectrophotometry for carminic acid with a more accurate and specific method, in order to prevent adulteration and ensure the authenticity of carmine. Because FDA usually refers to FCC standard with regard to food grade ingredients, FCC standard modernization is very important to ensure food safety. This research was funded by U.S. Pharmacopeia.

An HPLC method was developed to analyze the content of carminic acid in carmine. The HPLC method employed an Agilent ZORBAX SB-C18 column (15 cm x 4.6 mm, 3.5 μm). Column temperature was maintained at 35°C. Mobile phase consists of acetonitrile, methanol and 0.1% of trifluoroacetic acid in water (13:13:74, v/v). Flow rate is 0.8 mL/min. UV detector was set to be 494 nm. Carminic acid from DR. E (Lot: 20521) was used as a reference standard. Results of method validation proved the HPLC method is accurate and specific to analyze carminic acid. It was demonstrated that the HPLC method is able to differentiate carminic acid, ponceau 4R, and sudan I.

The HPLC method will be proposed to replace the existing spectrophotometry for carminic acid in order to fulfill the need of modernizing the existing FCC carmine standard.

Keywords: Food Identification, Food Science, Liquid Chromatography, Method Development
Application Code: Food Science
Methodology Code: Liquid Chromatography
The analysis of fatty acid methyl esters (FAME) is used for the characterization of the lipid fraction in foods, and is one of the most important applications in food nutrition analysis. GC columns for the analysis of FAMES must possess the necessary selectivity and efficiency for resolution and accurate quantification of the fatty acids that make up complex lipid classes. High polarity GC columns such as the highly-substituted cyanopropyl phases, HP-88, CP-Sil 88 and SP-2560, have historically being used for this purpose due to their capability to resolve positional geometric (cis/trans) FAME isomers. These columns, however, possess some disadvantages, such as longer analysis time, and significant amount of carbon chain overlap in the elution patterns that may lead to peak identification problems.

In this work, we evaluate the use of a low-content cyanopropyl phase for FAME analysis. DB-23, which is a 50% Cyanopropyl-methylpolysiloxane, has similar characteristics as the high-cyano content phases, including the important strong dipole-dipole interactions, needed to separate cis/trans isomers. Results demonstrates that even at 50% cyanopropyl content, this GC column phase is capable of separating challenging isomers such as C18:1 and C18:2 cis/trans, with the trans isomer eluting prior to cis isomer. In addition, our results indicate that FAMEs in the DB-23, are separated according to carbon number and degree of unsaturation, generating elution patterns typical of WAX-type phases. Finally, we demonstrate that routine analysis of common saturated and polyunsaturated FAMEs present in food samples, including milk fat, Omega’s 3 and 6, animal fat and vegetable oils, can be achieved under 8 minutes, with baseline resolution of critical pairs.

These findings suggest that fast routine analysis of FAMEs, without sacrificing resolution between geometric isomers, is possible when reducing the content of the cyanopropyl ligand in methylpolysiloxane phases.

Keywords: Food Science, Gas Chromatography, GC, GC Columns
Application Code: Food Science
Methodology Code: Gas Chromatography
Let's Analyze Beer: Determining Flavors and “Defects” in Beer by Headspace Trap/Gas Chromatography/Mass Spectrometry (HStrap/GC/MS)

Beer is a popular beverage produced by the fermentation of hopped malt extracted from barley and other grains. Some compounds have a positive effect on aroma (attributes) and some have a negative effect (defects). This presentation will focus on a new method that enables the quantification and characterization of flavors and defects, raw material investigation and competitive products of beer in one analysis using HS trap/GC/MS.

Classically, this analysis is performed on four separate detectors. This new method employs one detector to provide these solutions required for the production and the testing of beer. The outcome is a more cost effective, accurate means to ensure the validity and the quality control of product. Other benefits include enhanced productivity, attaining more information from a single analysis, and requiring less bench space.

The following experiments and results will be discussed.

- Quantitation of “defects”
- Characterization of several types of beers
- Fermentation profiling
- Analysis of raw materials
- Aging studies

Keywords: Flavor/Essential Oil, Food Science, Gas Chromatography/Mass Spectrometry, Headspace

Application Code: Food Science

Methodology Code: Gas Chromatography/Mass Spectrometry
Evaluation of a New Ultra Inert WAX GC Column for the Analysis of Fatty Acids, FAEEs and FAMEs

Primary Author: Gustavo Serrano Izaguirre
Agilent Technologies

Co-Author(s): Allen K. Vickers, Laura Provoost, Phil Stremple, Vanessa Abercrombie, Yun Zou

Abstract Text

GC Columns with Polyethylene Glycol (PEG) stationary phases are commonly used for analyzing compounds with polar functional groups, and are well suited for food, flavor and fragrances applications. A current challenge with traditional PEG phases, however, is the need to incorporate functional groups, such as nitroterephthalic acid, to separate challenging analytes like acidic organic compounds. These modifications, nevertheless, typically reduces column lifetime, maximum operating temperatures and are prone to react with some active analytes.

Continuing with our recent advances in Ultra Inert (UI) technology, we are introducing a new WAX UI phase, specifically designed for the analysis of fatty acids in free and ester forms. This new WAX phase (DB-FATWAX UI), delivers superior inertness, better long-term thermal stability and greater sensitivity than any other traditional WAX column. The excellent peak shapes obtained for acidic compounds, eliminate the need to use two separate GC columns for the analysis of free fatty acids (FFAs) and their esters in the same sample.

In this work, we present a variety of applications on the analysis of free fatty acids, fatty acid methyl esters (FAMEs) and fatty acid ethyl esters (FAEEs); including determination of volatile FFAs from dairy products, analysis of Omega 3 and Omega 6 per AOCS Ce 1b-89, and FFAs and FAMEs in complex mixtures with other organic acids. The use of DB-FATWAX UI for the analysis of FFA and naturally occurring FAEEs in distilled alcoholic beverages without sample pretreatment, is also discussed.

Keywords: Food Science, Gas Chromatography, GC Columns, GC-MS
Application Code: Food Science
Methodology Code: Gas Chromatography
The coffee industry is an important part of the global economy and its final product is one of the most consumed beverages in the world. As expected with commodities, there is a large amount of taste and flavor variation in coffee that is related to differences in the variety and geographical origin of the beans, storage and processing conditions, roasting conditions, and brewing conditions, among other factors. In addition to the expected variation, the aroma profile for coffee is quite complex and comprised of a large number of individual analytes. An understanding of these analytes can be helpful for quality control, process optimization, and also for providing information on flavors that direct consumers to their preferred styles. Non-targeted chemical analysis techniques, like gas chromatography with mass spectrometry (GC-MS) and headspace solid phase micro-extraction (HS-SPME), are well-suited for this type of work. Volatile and semi-volatile analytes were collected from coffee samples, separated, and detected, resulting in identification and relative quantification information for hundreds of analytes. Analytes of interest do not need to be determined prior to acquisition, so the data were generally characterized to investigate the samples and their differences. Comprehensive two-dimensional gas chromatography (GCxGC) increases peak capacity with an additional complementary separation dimension and was also explored here. With GCxGC, more analytes were separated and this additional analytical capability led to an improved understanding of these complex samples.

Abstract Text

The coffee industry is an important part of the global economy and its final product is one of the most consumed beverages in the world. As expected with commodities, there is a large amount of taste and flavor variation in coffee that is related to differences in the variety and geographical origin of the beans, storage and processing conditions, roasting conditions, and brewing conditions, among other factors. In addition to the expected variation, the aroma profile for coffee is quite complex and comprised of a large number of individual analytes. An understanding of these analytes can be helpful for quality control, process optimization, and also for providing information on flavors that direct consumers to their preferred styles. Non-targeted chemical analysis techniques, like gas chromatography with mass spectrometry (GC-MS) and headspace solid phase micro-extraction (HS-SPME), are well-suited for this type of work. Volatile and semi-volatile analytes were collected from coffee samples, separated, and detected, resulting in identification and relative quantification information for hundreds of analytes. Analytes of interest do not need to be determined prior to acquisition, so the data were generally characterized to investigate the samples and their differences. Comprehensive two-dimensional gas chromatography (GCxGC) increases peak capacity with an additional complementary separation dimension and was also explored here. With GCxGC, more analytes were separated and this additional analytical capability led to an improved understanding of these complex samples.
The quantitative analysis of flavor and fragrance samples is hindered by the number of compounds and the difficulty of obtaining suitable calibration standards. In lieu of accurate response curves, scientists are forced to estimate the concentration of analytes, potentially leading to concentration errors in excess of 100%. In this talk, we describe the use of the Polyarc® system coupled with a flame ionization detector (FID) to accurately quantify complex fragrance mixtures without using calibration standards. Identification and quantification are obtained through a single injection using a post-column MS/Polyarc-FID split. The result is improved quality control, data integrity and production reliability.

Keywords: Calibration, Flavor/Essential Oil, Food Science, Gas Chromatography
Application Code: Food Science
Methodology Code: Gas Chromatography/Mass Spectrometry
Milk powder is the second most likely food item being in the risk of adulteration after olive oil. Adulterants in milk mainly include addition of vegetable protein, milk from different species, addition of whey as economically motivated some adulterants are too harmful having serious adverse health effect. Methods which can detect multiple adulterants & contaminants & or establish food authenticity are needed. Studies were undertaken to develop a specific method to detect milk adulteration by amino acid fingerprint technique. Authentic skim milk powder samples were obtained and analyzed for amino acid composition with and without spike with different chemicals which are potential protein or nitrogen sources for milk adulteration. The developed method of amino acid identification score determination could successfully differentiate between the authentic and adulterated milk samples irrespective of the method of amino acid analysis.

Keywords: Food Science, Forensic Chemistry, HPLC, Liquid Chromatography

Application Code: Food Identification

Methodology Code: Liquid Chromatography
The increasing complexity of protein therapeutics over the last several decades has required the analytical field to keep pace with better techniques for separating, detecting and characterizing the different variant structures that arise during production. Manufacturers and regulatory agencies require identification and quantitation of the different variants as many of them can adversely affect the efficacy and safety of protein therapeutics. Chromatographic methods are commonly employed to separate and analyze the components of proteins in process and as final production formulation. Specifically, cation exchange chromatography has become a method of choice for separating protein charge variants (e.g., lysine truncation, oxidation, glycosylation, etc.). This technique is commonly applied using a salt gradient (from low to high salt concentration) or a pH gradient (from low to high pH) with acidic (more negatively charged) variants eluting prior to the predominant structural peak and basic (more positively charged) variants eluting afterward.

Here, we report on the development of a weak cation exchange column using a 5\(\text{\textmu}m\) divinylbenzene-based resin particle. The resin is coated with a hydrophilic polymer layer followed by grafting of poly(acrylic acid) to provide weak cation exchange functionality. General considerations for good chromatography include analyte selectivity, peak shape and resolution, and recovery and carryover. Additionally, the ability to achieve separations over shorter time periods enables high throughput analysis, which is critical during the development stages of new protein therapeutics. We discuss the performance of this 5\(\text{\textmu}m\) WCX resin with regards to these attributes using simple protein analytes and monoclonal antibodies (mAbs). Both salt and pH gradients will also be presented for the analysis of mAbs.

Keywords: Bioanalytical, Biopharmaceutical, HPLC, Protein
Application Code: Bioanalytical
Methodology Code: Liquid Chromatography
Classical solvent gradients permit high-resolution separations of complex mixtures in liquid chromatography. They lead to rapid separations and contribute to the thermodynamic compression of the peak width depending on how sensitive the analyte retention is to the eluent strength [1]. Faster gradient runs have been realized by combining uniform temperature gradients with usual solvent gradients [2]. Yet, such a gradient combination does not participate effectively to the phenomenon of peak compression because the temperature remains always uniform along the peak width. There is place for improvement by design properly this solvent-temperature gradient combination.

In this presentation, a new class of gradients is proposed for microfluidic columns (i.d. smaller than 500 [micro]m) : the classical solvent gradient is combined with a non-stationary (finite linear velocity) and a non-uniform temperature (finite steepness) gradient. Accordingly, both the solvent composition and the temperature are now participating to peak compression. The advantages of this gradient in terms of peak capacity per unit time are demonstrated from a theoretical viewpoint [3]. The calculation results are guiding the users towards the optimum speed and steepness of the temperature gradient to be applied in chromatography. A series of prototype temperature devices recreating the conditions of a traversing temperature gradient are built and their performance presented in details. Finally, a 300 [micro]m x 100 mm microfluidic column packed with 3.5 [micro]m XSelect HSST3 particles is tested in order to confirm the expected gain in gradient resolution of polycyclic aromatic hydrocarbons and peptides when combining dynamically solvent and temperature gradients.

We have recently reported our improved online separation and detection of microdialysis neurotransmitter dopamine (DA) at 1 min time resolution, which allowed for a more detailed observation of extracellular dopamine concentration under basal and stimulated conditions in awake rats. However, even faster time resolution is needed to completely understand psychological and physiological neurochemical responses. Thus, we have further optimized our online microdialysis system, enabling online monitor of striatal dopamine at 45 s time resolution. We capitalized this faster time resolution to design a method whereas a No-Net-Flux (NNF) experiment, which is typically done in 2-4 hours, can be performed in 18 minutes. Three retrodialysis DA concentrations (50, 200 and 600 nM) were perfused in the striatum of an awake rat, at 4.5 min/concentration. Dialysate DA concentration was measured online and used to calculate the extracellular concentration of DA as well as microdialysis probe extraction fraction. Although the NNF assumptions are correct for an in vitro experiment, that a constant retrodialysis concentration results in a plateau dialysate concentration, we hypothesize that the DA concentration in the rat dialysate instead reaches a maximum then slowly decreases. We believe this is due to an increase in the reuptake of DA in response to a higher extracellular concentration caused by the DA perfusion. Our probes achieve a consistent extraction fraction of approximately 15% in vitro. We expect similar extraction fraction in vivo. The rate of increase and decrease of the dialysate DA concentration can be measured experimentally and give a measurement of DA diffusion across the membrane and extracellular space as well as rate of increase in DA reuptake.

Keywords: Bioanalytical, Capillary LC, HPLC, Neurochemistry
Application Code: Neurochemistry
Methodology Code: Liquid Chromatography
A versatile bench-top chromatograph has proven useful for protein and other polymer separations. The Rotify centrifuge rotates a membrane-bearing rotor that has liquid flow through a spiral channel on the top and flow in the opposite direction below the MW cut-off membrane. A protein sample passes into the rotor and all low MW entities pass through the membrane and the individual proteins continue traveling along and are precipitated at individual rates according to a salt or solvent gradient applied. The elution of the separated proteins or polymers come out the other end collected in fractions. Examples of protein isolation and purification in one step out of complex extracts/solutions have been described and the device is useful for antibody purification, as well as purification of conjugates, faster than dialysis. Here we report a method of extending over a long time, the ammonium sulfate gradient at a low RPM and being able to purify some very high MW proteins. Phycoerythrin B from red seaweed is a light harvesting pigment-protein complex of \((\bullet\bullet)^6\) with a MW of 266 kDa. In the purified fractions the ratio of absorbance at 565 nm to that of 280 nm was 5.0, much higher than previously published value of 3.7. The purified fraction had the same mobility in PAGE as the major component in the extract. An example of a virus-like particle (VPL) has been investigated. The Adeno-viral vector serotype 9 with green fluorescent protein (AV9GFP) was precipitated from cell supernatant with PEG and ran in the Rotify (prototype instrument) with a faster salt gradient; the VLPs were highly retained and pumped out in the contents. The analysis by SDS-PAGE showed a little less purity comparable to the results of conventional fractionation by ultra-centrifugation, but a good result for a one-step process and a higher recovery. An ammonium sulfate precipitate was separated in the long gradient and the results will be presented.
The research on oligosaccharides is growing and gaining in importance at a phenomenal pace. The efforts to understand their bioactivity and to develop new products based on oligosaccharides in biotherapeutics and food industry require effective and reliable analytical techniques for determination of oligosaccharides in various samples. Here we present a new analytical method based on the use of dual electrolytic eluent generation platform and high performance anion-exchange liquid chromatography (HPAE) in both conventional and capillary separation formats for the analysis of oligosaccharides. The system consists of one eluent generator producing methanesulfonic acid (MSA) connected in series with a second eluent generator producing potassium hydroxide (KOH). Through manipulating the concentration output of both the eluent generators, chromatographic performance comparable to that obtained using the conventional sodium acetate/sodium hydroxide (NaOAc/NaOH) eluent system is achieved using the electrolytically-generated potassium methanesulfonate/potassium hydroxide (KMSA/KOH) eluent. This platform overcomes the various drawbacks associated with manually-prepared NaOAc/NaOH eluent, utilizes deionized water as the only carrier stream through a single isocratic pump, and offers an easy-of-use, simplified operation solution for oligosaccharides profiling with increased precision and accuracy. Several examples of using this new analytical techniques for analyzing oligosaccharides will be presented.
A robust and customizable stationary phase is desired to enhance the separation and sensitivity of the on-line monitoring of neurotransmitters using HPLC. Polymeric coated stationary phases for HPLC have been made to create more stable silica based stationary phases. These are often made with siloxane polymers, or a hyper-crosslinked aromatic polymer created using the Friedel-Crafts reaction. These reactions are done using precise reaction conditions, often with numerous tedious steps. This limits the easy customization of the stationary phase to produce the column chemistry needed for challenging separations. The thiol-yne reaction provides a robust, easy synthesis of highly crosslinked polymers. We have used this click chemistry reaction to synthesize polymer coated stationary phases with differing characteristics without any catalysts that can harm chromatographic performance. To create a simple polymer coated material, porous silica particles were thiol functionalized using 3-mercaptopropyltriethoxysilane. 1,4-diethynylbenzene (DEB) was then attached to the thiol functionalized silica. The alkyne substituted ligands were cross-linked using 1,6-hexanediethiol and a small amount of DEB. A mixed mode strong cation exchange (SCX) stationary phase was also made by the addition of sodium 3-mercaptopropanesulfonate as a reagent during the crosslinking step. The stationary phases were characterized using the Tanaka Test and other methods. The added propyl groups on the SCX phase help increase its retention of pentylbenezene compared to the polymer only phase. The SCX also has added ion exchange capacity, and positively charged solutes’ retention can be changed by varying the ionic strength of the mobile phase. This allows positively charged monoamine neurotransmitters to be analyzed without using any ion-pairing surfactant, helping to simplify their analysis.

Keywords: Chromatography, HPLC, HPLC Columns, Liquid Chromatography
Application Code: Bioanalytical
Methodology Code: Liquid Chromatography
Studying dynamic concentration changes of neurotransmitters within the functioning brain is important to better understand their pathways and role in disease. Online sampling allows for the direct introduction of sample onto the HPLC system using a microdialysis probe. However, the relationship between flow rate and probe recovery limits the moles of sample loaded onto the column per injection for a given sampling time. Temporal resolution goals set below one-minute challenge the study of these molecules due to low concentration sensitivity and resolution in the dialysate matrix. Because of this, online separation conditions at this time scale must be optimized individually for dopamine (DA) or serotonin (5-HT), preventing simultaneous basal monitoring.

In this work, we apply our temperature-assisted solute focusing (TASF) devices to achieve simultaneous one-minute measurements of DA and 5-HT. An array of 1 cm\textsuperscript{2} thermoelectric cooling elements (TECs) capable of independent and rapid temperature changes, control the separation temperature of the capillary column to modulate solute retention. By operating this temperature programming near the end of the column, DA retention can be selectively controlled to shift the elution time after the early eluting interferences and closer to 5-HT, while maintaining one-minute temporal resolution of both solutes.

The advantage of integrating TASF into fast online separations of neurotransmitters is demonstrated through in vivo studies using animal models. Our results are supported by theoretical simulations which also provide a tool used for method development to determine optimum separation conditions. This method was successful in monitoring the extracellular concentration response of DA and 5-HT to K\textsuperscript{+} stimulations through retrodialysis with sub-minute temporal resolution, while increasing the sensitivity of basal DA relative to isothermal separations.

**Keywords:** Bioanalytical, HPLC, Liquid Chromatography, Neurochemistry

**Application Code:** Bioanalytical

**Methodology Code:** Liquid Chromatography
Advances in HPLC designs which utilize higher pressures for smaller inner diameter columns have created analyte recovery issues caused by greater wall contact time. The loss of analytes caused by surface activity has been a common issue with the small sample sizes in GC for years. Now, with modern narrow-bore columns, the loss of sample is a major hurdle to overcome in UHPLC. Successful chromatography of proteins, polar and trace compounds created during new drug discovery efforts require bio-inert substrates in the sample flow path. Traditional rugged and high-strength materials such as stainless steel are not bio-inert, while inert materials such as PEEK cannot handle the pressure demands of UHPLC.

This presentation will compare stainless steel, PEEK and Dursan-coated stainless steel with respect to chromatographic performance of difficult-to-analyze compounds. Discussion will also center on the application flexibility of the coating, particularly to all parts of the flow path from frit to pump. Finally, to demonstrate durability, data on exposure to both caustic and acidic environments will be presented.

**Keywords:** Bioanalytical, Capillary LC, Drug Discovery, HPLC
**Application Code:** Bioanalytical
**Methodology Code:** Liquid Chromatography
Neutral lipids constitute as an important class of cellular lipid molecules. They play critical roles that drive energy production and storage, membrane fluidity, and signal transduction in an organism. Shotgun lipid analysis, primarily using electrospray ionization mass spectrometry (ESI-MS), has become an indispensable tool for lipidomics. However, neutral lipids such as sterols and glycerides often go under-detected by ESI-MS because they lack easily-ionizable charge moieties. Charge derivatization has been demonstrated as a successful strategy to boost neutral lipids response in ESI. Established methods typically require multi-step procedures for derivatization and sample preparation before ESI-MS; the overall process typically takes several hours. Herein, we exploit a one-pot photochemical derivatization based on tagging unsaturated neutral lipids with thiol reagent containing a charge group. This approach significantly enhances ionization of neutral lipids in ESI, leading to high sensitivity (pM range) and wide coverage of lipid species in complicated mixtures. This method can be applied to small sample volume (1 μL) and doesn’t require extensive sample extraction procedure or hydrolysis. In addition, the photoreaction was fast (less than 1 minute) by using an easily constructible photo-microreactor system. Tandem MS/MS, employing lipid class specific neutral loss and precursor ion scanning, was incorporated with this study for improved structural characterization and multiplexed quantification of individual neutral lipid classes. The applicability of this method for a fast and comprehensive characterization of neutral lipidome is illustrated on samples of human plasma. Development of this efficient strategy could further facilitate our understanding the role of neutral lipids in biological systems.

Financial support from NIHGM R01GM118484 is greatly appreciated. Y.X acknowledges ASMS research award for supporting research on radical ion chemistry studies.
Coated Blade Spray (CBS) is a SPME-based technology designed for the enrichment of analytes of interest from complex sample matrices, which can be directly coupled with mass spectrometry (MS) instruments for rapid quantitative/qualitative analysis. Unlike direct-sample-to-MS approaches, CBS provides a cleaner extract which allows for long-term operation of the instrument with minimal maintenance and reliable quantification. The main goal of this work is to describe most recent advances on CBS-technology that shift paradigms related to the direct coupling of samples to MS. First, we present a CBS-autosampler that allows for processing of up to 96-samples simultaneously and the subsequent unsupervised MS-event of each CBS. This technology was assessed for the quantitative determination of drugs in urine, blood and plasma samples. Model analytes with a wide variety of physical-chemical and protein binding properties, including doping agents (e.g. clenbuterol), pain-management drugs (e.g. fentanyl), and therapeutic-drugs (e.g. tacrolimus) were selected for this study. Our results demonstrated that CBS can provide satisfactory linearity over 3 orders of magnitude (pg/mL to ng/mL) and great accuracy (85-120%) for the majority of the analytes selected. Second, we introduce a ground-breaking strategy that allows for the concomitant analysis of multiple target compounds (e.g. immunosuppressive, antifungal and pain-panel drugs) from a blood droplet. Besides, we describe diverse on-coating derivatization methodologies developed to enhance limits-of-quantitation for targeted analytes with poor ionization efficiency/high-instrumental background. Finally, we explore this technology’s suitability for the on-site rapid molecular profiling of complex matrices and quasi-real time identification via on-line recognition software.

Keywords: Bioanalytical, Clinical Chemistry, Mass Spectrometry, SPME
Application Code: Bioanalytical
Methodology Code: Mass Spectrometry
Tranexamic acid (TXA) is a drug used for antifibrinolytic therapy in a range of applications including high risk cardiac surgery. Unfortunately, it has proved difficult to maintain plasma concentrations of this drug within its therapeutic range during treatment which can lead to post-operative seizures especially for patients suffering from renal insufficiency. It is therefore important to develop efficient methodologies to monitor this drug more closely over the course of administration to better make corrections to the dosing schedule and in effect, tailor the treatment.

In this context, the use of new and simple technologies such the microfluidic open interface (MOI) through flow isolated desorption volume emerge as an interface for the direct coupling of biocompatible solid-phase microextraction to tandem mass spectrometry (Bio-SPME-MOI-MS/MS).

In this work, a cross validation between Bio-SPME-MOI-MS/MS and Bio-SPME-LC-MS/MS is conducted. Patient sample preparation was done via Bio-SPME using a hydrophilic-lipophilic balance (HLB) type coating. It must be noted that for MOI-MS/MS 4 mm Bio-SPME fibers were enough to reach the required LOQ while for the LC-MS/MS method the configuration that provided the best result was the blade. This methodology reduced analysis time from 10 minutes by way of LC to just 20 seconds per sample while keeping the same sensitivity. Linearity, precision and, LOQ were assessed for the techniques with values of r2>0.99, 15% and 10 ppm, respectively. The Bland-Altman plot of data pairs (n = 63) of Bio-SPME-MOI-MS/MS and Bio-SPME-LC-MS/MS showed that a 92.1% of the data fits within the acceptable range (interval of confidence ± 1.96 SD). Furthermore, the Deming regression gave a slope of 0.984, which is very close to the identity. These results demonstrate the suitability of this technology not only for high-throughput determination of TXA from plasma but also for different clinical applications where the time is a crucial variable.
Over the last few decades aptamers have rivalled antibodies as the leading affinity reagents to a wide variety of targets. Aptamers were first discovered using the selection process known as Systematic Evolution of Ligands by Exponential Enrichment (SELEX). In this process oligonucleotides are selected from a combinatorial library for high affinity, highly selective binding to a specific target. A variety of aptamers have been discovered using SELEX but it does have some limitations which hinder the discovery of potential aptamers that form multi-tier G-quadruplexes (G4). These sequences are found throughout the human genome and warrant investigation as potential aptamers. We have developed a new genome-inspired, reverse selection approach that allows us to explore these secondary structures. In the reverse selection approach, specific DNA sequences from the human genome are used for affinity capture of proteins from natural pools such as nuclear protein extracts. The nuclear extracts are incubated with streptavidin magnetic beads coated with biotin-modified G4 oligonucleotides or control oligonucleotides. A rapid screen for affinity captured proteins specific for G4 DNA is performed using MALDI-TOF MS. If affinity capture is established, the captured proteins are separated using gel electrophoresis. Bands specific for G4 DNA are excised and analyzed using LC-MS/MS to produce a list of possible protein matches. This list is used to guide the selection of proteins for further examination using Western blot. Here we will present our results for genomic-G4 forming sequences from the promoter regions of the human oncogenes c-myc, Rb and VEGF as potential aptamers towards proteins from nuclear extracts from basal breast cancer cell line MDA-MB-468.

Keywords: Bioanalytical, Genomics, Nucleic Acids, Protein
Application Code: Bioanalytical
Methodology Code: Mass Spectrometry
In recent years, mass spectrometry has been applied to large molecular systems. Fragmentation of large protein and nucleoprotein complexes by solution disruption and by collision induced dissociation with gaseous targets provides some structural information but does not always provide full connectivity information and information on relative interface strengths within the complex. The Wysocki lab has developed the technique of surface induced dissociation, coupled with ion mobility dissociation and high resolution mass spectrometry as a tool to fragment protein and nucleoprotein complexes in a structurally diagnostic way. This talk will highlight progress made with a variety of instrument types (QTOF, Orbitrap, ICR). Challenges still faced in the development of SID in these instruments and possible solutions will be described. The use of complementary mass spectrometry approaches for overall structural characterization of macromolecular protein complexes will be highlighted.
Nowadays, technologies that efficiently integrate the sampling and sample preparation step with the direct introduction to mass spectrometry (MS) can generate a great impact in a vast variety of fields such as clinical, environmental, and food sciences. In this study, a novel interface that allows the direct coupling of Bio-SPME devices to MS via a microfluidic open interface (MOI) is presented. This technology, which operates under the concept of flow isolated desorption volume, consists of an open-to-ambient desorption chamber (V•7 µL) where the extracted analytes are delivered. Subsequently, compounds of interest are transported to the ionization source by means of the self-aspiration process intrinsic of these interfaces. Thus, any ionization technology that provides a reliable and constant suction, such as electrospray ionization (ESI), atmospheric chemical ionization (APCI) or inductively coupled plasma ionization (ICP), can be hyphenated to MOI. Numerical simulations provided detailed understanding of the fluidics inside the interface and helped optimizing the system. The analytical workflow in SPME-MOI-MS consisted in the direct immersion of the SPME fiber into the matrix to extract/enrich the analytes for short times, followed by a rinsing step with water. Then, the fiber is inserted into the MOI to desorb the compounds of interest. Finally, the volume contained in the chamber is drained and the plug of analytes is moved towards the electrospray needle for ionization and direct introduction to MS. As a proof-of-concept, the fast determination of immunosuppressive drugs (e.g. tacrolimus, cyclosporine, sirolimus, and everolimus) from 100 µL of whole blood was assessed. Limits of quantitation in the sub-ppb range were obtained for all studied compounds. Good linearity (r² •0.99) and excellent precision, with (8%) and without (14%) internal standard correction, were attained for a fix-hematocrit level.

Keywords: Flow Injection Analysis, SPME, Tandem Mass Spec, Biomedical
Application Code: Biomedical
Methodology Code: Mass Spectrometry
Tears play blood-like roles for the avascular cornea and the chemical composition of tears can be related to corneal health. In particular, the composition analysis of tears will help in the identification of corneal complications that develop for diabetic patients. However, there remain significant challenges in the collection and analysis of tear chemical content. The purpose of this study is to demonstrate the applicability of a MALDI-TOF/TOF for qualitative and quantitative analysis of tear amino acids and other small molecules. Our laboratory has previously demonstrated that tear fluid can be collected with sterile standardized phenol-red threads (PTR) non-invasively. Elutant from the threads in this study were analyzed with 4800 or 4700 plus MALDI TOF/TOF analyzer calibrated by red phosphorus. Several amino acids as well as others small molecules were detected and identified by mass determination. Quantitation of select amino acids was studied using ion counting and comparison to standard amino acids linear calibration curves. Preliminary assessment of human and mouse tears demonstrates method applicability. Tears were collected from both eyes of mice and humans by placing a PRT in the conjunctiva sac for 20 sec. The extraction of tear content from the PRT was optimized with several organic solvents and shows dichloromethane to provide ideal capabilities. Tear elutant ionization was studied with various matrices, demonstrating the utility of -cyano-4-hydroxycinnamic acid. The resultant spectra of both human and mouse tear films showed a large number of peaks of both amino acids and steroid molecules. Linear calibrations with standards allowed preliminary assessment of tear amino acid concentrations in mice tears. Our results demonstrate that MALDI TOF/TOF MS is an effective tool for analyzing tear samples collected by PTR.

Keywords: Amino Acids, Extraction, Identification, Sample Preparation
Application Code: Biomedical
Methodology Code: Mass Spectrometry
A Robust Method for Ultra-High Resolution MS Elemental Composition Determination

High resolution accurate mass spectrometry is an important tool for the identification of unknown organic compounds. Normal practice is to calculate all realistic chemical formula within the confidence limit of the instrument (e.g. five part-per-million about the monoisotopic peak). This rarely, if at all, provides a unique elemental composition (formula ID) but instead produces a list of formula candidates which must be carefully evaluated to provide confident ID.

One approach to improving formula ID is to take advantage of the unique isotope patterns observed at the A+1 and A+2 lines at resolutions around R = 240,000, readily attainable on the newer Orbitrap MS or conventional FT ICR MS. For example, the shape of the A+2 line of the unknown compound provides a highly unique pattern with good resolution of the isotope contributions from [15N]2, [13C] + [15N], [18O], [13C]2, and [13C] + [2H]. These patterns provide a “fingerprint” which can be used to identify a unique elemental composition if only it can be accurately compared to calculated patterns. Unfortunately, two factors limit our ability to perform this comparison. The first is the fact that the mass spec instrument lineshape is undefined making accurate comparison against calculated spectra problematic. Second, instrument related distortions like space charge effects including coalescence can dramatically distort these fingerprints, both in mass position and intensity, further complicating the ability to confidently and accurately compare between measured and calculated mass spectrum.

In this presentation, we will present a novel method to address these problems which provides significantly improved confidence in formula ID. The method involves (1) the calibration of the instrument lineshape as derived from the monoisotopic peak and (2) careful control and automatic selection of the optimum ion population in the Orbitrap to reduce/minimize instrument related distortions.

Keywords: Liquid Chromatography/Mass Spectroscopy, Mass Spectrometry, Pharmaceutical
Application Code: Pharmaceutical
Methodology Code: Mass Spectrometry
West Nile virus (WNV) is an arthropod-borne flavivirus most commonly transmitted to humans by female Culex mosquitoes. Infection can result in severe neurological symptoms such as meningitis or encephalitis, and 46,000 cases have been reported in the US alone since 1999. Current studies of WNV spread predict increasing rates of transmission as global temperatures increase. Therefore, there is a need to develop point-of-care techniques that allow for increased surveillance of WNV spread. This is particularly important as current detection methods, such as real-time quantitative polymerase chain reaction and plaque reduction neutralization assays are insufficient for use in developing countries in terms of assay cost, speed and resource requirements. In this work, we present a microfluidic paper-based analytical device (PAD) coupled with electrochemical impedance spectroscopy for detection of WNV and biotin-streptavidin nanoparticles. The assay is based on the specific binding of WNV to a 4G2 panflavivirus antibody modified Au microelectrode (Figure 1a), and sensing through an increase in ferricyanide charge transfer resistance (Rct) on binding (Figure 1b – size of semicircle). Use of a 2-layer, self-pumping PAD facilitates pre-concentration of WNV from a sample onto the modified electrode (Figure 1c), resulting in a detection limit of 10.2 WNV particles in a 50 µL aliquot. Importantly, we observe no increase in Rct for an excess of a non-specific target (Sindbis virus), and no binding of the WNV to unmodified electrodes (from scanning electron microscopy). Finally, the method scope is demonstrated through quantification of a wide range of streptavidin nanoparticle sizes (40 nm – 50 nm). This detection motif represents a cheaper, faster, simpler and sensitive point-of-care alternative to current methodologies, and is adaptable towards a wide range of viruses and other analytes.

Keywords: Bioanalytical, Electrochemistry, Lab-on-a-Chip/Microfluidics, Paper/Pulp
Application Code: Bioanalytical
Methodology Code: Microfluidics/Lab-on-a-Chip
A PMMA/Paper Hybrid CD-Like Microfluidic SpinChip Integrated with DNA-Functionalized Graphene Oxide Nanosensors for multiplex qLAMP Detection

Although the recently-introduced loop-mediated isothermal amplification (LAMP) is gaining increasing attention for biological applications, its applications are significantly circumscribed due to the lack of effective methods for quantitative multiplex LAMP (mLAMP) detection. Herein, we developed a poly(methyl methacrylate) (PMMA)/paper hybrid CD-like microfluidic SpinChip integrated with DNA-functionalized graphene oxide (GO) nanosensors for simple multiplex quantitative LAMP detection (µ-mqLAMP). Multiple DNA targets were isothermally amplified in a single microzone (i.e. mLAMP). The microfluidic SpinChip enabled amplicons and other reagents to be simply manipulated to tens of detection microzones by rotating the top PMMA plate manually, without using any complicated pneumatic values. Amplified DNA targets were further identified and quantified with high specificity by the integrated DNA capture probe-functionalized GO nanosensors in different detection microzones on the chip. Paper inside the detection microzones facilitated the integration of nanosensors, thus avoiding complicated surface modifications. We demonstrated the proof of concept by testing two main pathogens that cause the serious bacterial meningitis, Neisseria meningitidis (N. meningitidis) and Streptococcus pneumoniae (S. pneumoniae). The limits of detection (LODs) of 6 and 12 DNA copies per assay for N. meningitidis and S. pneumoniae were obtained in about 1 h. No washing or purification steps were needed during the whole assay process. This study for the first time provided a simple and effective microfluidic approach for multiplex quantitative LAMP (i.e. mqLAMP) detection.

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Keywords: Bioanalytical, Biomedical, Biosensors, Lab-on-a-Chip/Microfluidics
Application Code: Bioanalytical
Methodology Code: Microfluidics/Lab-on-a-Chip
A High-Sensitivity Immunoassay in a Reusable Paper/Polymer Hybrid Plug-and-Play Microfluidic Platform

Conventional colorimetric enzyme linked immunosorbent assay (ELISA) consumes a large amount of sample, and requires overnight incubation and costly equipment, but the detection sensitivity is low. Herein, a reusable, cost-effective, and eco-friendly polymer/paper hybrid plug-and-play (PnP) device for high-sensitivity immunoassay through analyte enrichment has been developed. The device consists of three poly(methyl methacrylate) (PMMA) layers with multiple slots where SU-8 patterned paper-strips can be inserted. The PMMA-framework provides well-controlled conditions for reproducible results while low-cost paper can rapidly immobilize proteins avoiding complicated surface-modifications. Paper-strips can be plugged into the device to perform the bioassay in a PnP format. Back-and-forth passing of samples through the porous 3D paper at a certain flow rate from a syringe pump assures maximum pre-concentration of the analyte. The process dramatically decreases the incubation time from several hours to a few minutes and also increases the detection sensitivity. After the assay, the result can be viewed by the naked eyes or scanned through a common desktop scanner for quantitative analysis. The paper-strips can be replaced after the assay so that the PMMA-framework can be reused. The limits of detection of 200 pg/mL for immunoglobulin G and 270 pg/mL of hepatitis B surface antigen were observed, which is at least 10-folds more sensitive than commercial ELISA. In addition, dynamic linearity range was three-orders of magnitude wider than the paper-based device. This reusable device can be used for low-cost and ultrasensitive detection of amount-limited samples of infectious diseases and cancer, especially in resource-limited settings.

Abstract Text

Keywords: Bioanalytical, Immunoassay, Lab-on-a-Chip/Microfluidics, Polymers & Plastics

Application Code: Bioanalytical

Methodology Code: Microfluidics/Lab-on-a-Chip
The analysis of DNA is an important part of many fields including forensics, medicine and biology. While separation of DNA by size using sieving gels is straightforward, the separation of single stranded DNA of the same length by sequence is not as simple and usually requires sequencing the DNA strands of interest which can be costly and time consuming. Separation of DNA by sequence could provide important information about genetic diversity and environmental responses in microbial communities and biofilms, and about genetic mutations and variants, that would not be available from length-base separation alone. Our group has previously demonstrated a technique for separating DNA by sequence using capillary zone electrophoresis with high salt buffers. We have been able to separate 76-mers and 15-mers of DNA which differed by only one or two bases. In our current work we are now taking what we learned from the CE system and applying it to microchip electrophoresis. In the CE system it would take on the order of an hour to achieve separation. The separation channel on the microfluidic chip is much shorter than the capillary and therefore cannot achieve the same separation using regular CZE methods. To accomplish the separation by sequence in the microchip setup we have introduced a guanosine based gel to slow sample migration and improve separation. The guanosine gel also requires a high concentration of salt in order to form the gel, which makes it an ideal choice for the gel phase to help the separation of DNA by sequence. At the same time, the guanosine gel does not act as a sieving gel and so separation is solely by sequence under the high salt conditions. The goal is to create a two dimensional microfluidic chip to separate DNA first by size then by sequence.
Employing mass spectrometry based detection provides the potential to broaden the applications of droplet microfluidics. By mass spectrometry, multiple analytes can be monitored simultaneously, without the need for optical labels. Electrospray ionization-mass spectrometry (ESI-MS) has been applied for the analysis of droplet samples, but the formation of gas phase analyte ions can be suppressed when samples contain ESI unfriendly matrices. One way to address this problem is by reducing flow rates below 1000 nL/min, where marked increases in matrix tolerance can be observed. Our work has involved applying “nano”ESI-MS to droplet samples containing suppressing matrices. Droplets segmented by an immiscible fluorous carrier phase are flowed into capillary spray emitters that have been pulled to 15 or 30 µm inner diameter. 1.25-1.75 kV is applied to a conductive coating on the exterior of the emitter. For our first application, the levels of the neurotransmitters glutamate, GABA, and acetylcholine in a rat striatum were monitored by nESI-MS. Sampling was achieved by a microfabricated push-pull probe, and the sample stream was segmented into 3-5 nL droplets to preserve temporal resolution. Off-line nESI-MS analysis of the droplets was performed at 50 nL/min. Respective LODs of 94.8 nM, 34.6 nM, and 2.51 nM were found in-vitro, with linear calibration ranges that encompass expected experimental concentrations. nESI-MS is also being explored for the high-throughput evaluation of enzyme variants. 250 pL droplets were formed from in-vitro protein expression solution containing 100 µM substrate and product of our transaminase enzyme of interest. Distinct droplet-by-droplet signal was seen at flow rates ranging from 75-500 nL/min, up to a throughput of 7 Hz.

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Keywords: Bioanalytical, Electrospray, High Throughput Chemical Analysis, Lab-on-a-Chip/Microfluidics

Application Code: Bioanalytical

Methodology Code: Microfluidics/Lab-on-a-Chip
Microdialysis (MD) is a minimally invasive sampling technique that has been widely used for various in vitro and in vivo applications including biotechnology investigations. In majority of these applications the analysis of the microdialysis samples is performed off-line. For continuous online monitoring, MD sampling can be directly coupled to microchip electrophoresis (ME) separation devices. To date several coupling methods have been described for direct integration of MD sampling with ME. Most of these techniques use irreversible sealing of microdialysis-microchip interface to the substrate so that the device can withstand hydrodynamic flow. In the present work, a simple reversible sealing method to couple microdialysis sampling with microchip electrophoresis with electrochemical (EC) detection is described. To hold the microdialysis probe outlet in a fixed position at the MD-ME interface, a stainless-steel tube connected to a thicker PDMS block that is irreversibly bonded to the microchip is used. This method helps to obtain fast and accurate microchannel/electrode alignment for on-line MD-ME-EC analysis.

The device consists of a double t PDMS/glass hybrid microchip with a pyrolyzed photoresist carbon electrode. Long term stability of reversibly sealed MD-ME interface for on-line analysis was tested by monitoring the conversion of L-DOPA into dopamine in homogenized rat brain. The separation of 3-O-MD, L-DOPA, N-Tyr, HVA, DOPAC, and dopamine was achieved in less than 100s in vitro. The developed MD-ME-EC device is currently been employed on-line to monitor the transport of L-DOPA across the blood-brain barrier, its conversion to dopamine, and subsequent metabolism in vivo in an anesthetized rat.

Keywords: Biosensors, Electrochemistry, Electrophoresis, On-line
Application Code: Bioanalytical
Methodology Code: Microfluidics/Lab-on-a-Chip
A New Bar-Chart Microfluidic Chip Driven by Nanoparticle-Mediated Photothermal Effects for Point-of-Care Immunosensing

Wan Zhou
University of Texas at El Paso

Guanglei Fu, T Sanjay Sharma, Xiujun (James) Li

The volumetric bar-chart microfluidic chips (V-Chips), mostly driven by gases generated via chemical reactions, provide a simple yet powerful platform for visual biochemical quantitation. But they are faced with their instinct disadvantages, such as complicated fabrication procedures, interferences from environmental elements, and low reproducibility. The nanoparticle-mediated photothermal effect, as a promising tool to provide the stable driving force, has never been investigated in bar-chart microfluidic chips, especially for point-of-care (POC) analysis. Herein, we introduced a new nanoparticle-mediated photothermal effects–driven bar-chart microchip (PT-Chip) for visual quantitative immnosensing. In this strategy, iron oxide nanoparticles involved in a typical sandwich immunoassay were converted to Prussian blue nanoparticles (PB NPs), a near-infrared (NIR) photothermal agent, which were exploited to generate heat under the laser irradiation. The quantitation of biomolecules was achieved by visually reading the colored flow distance on the PT-Chip, without the aid of any bulky and expensive instruments. This PT-Chip was employed to detect the prostate-specific antigen (PSA) with high specificity and sensitivity, with the limit of detection of 1.0 ng/mL, and high reproducibility of 94.7%. The PT-Chip was further validated by testing human serum samples and whole blood samples, with acceptable analytical recoveries in the range from 89.1% to 92.5%. The novel introduction of nanomaterial-mediated photothermal effects into bar-chart chips opens a new horizon of bar-chart microfluidic devices for broad applications at the point of care. Financial support from NIH, NSF-PREM, MCA Foundation, UT STARS Award, MRAP, IDR, and URI Award from UTEP is gratefully acknowledged.

Bioanalytical, Immunoassay, Lab-on-a-Chip/Microfluidics, Nanotechnology

Bioanalytical

Microfluidics/Lab-on-a-Chip
Microchip electrophoresis integrated with amperometric detection (ME-EC) has been shown to be applicable for the separation and detection of both pro-oxidants and antioxidants. The limits of detection (LODs) for ME-EC is generally fall within sub micromolar range and therefore, not adequate to measure electroactive compounds present at sub micromolar concentrations.

Fluorescence detection has been utilized as an alternative detection technique for the ME and that offers picomolar LODs. Derivatization is generally required and that can leads to selectivity and sensitivity issues. Alternatively, a bipolar electrode can be used to transform the current to an optical signal such as fluorescence. In this study, a novel detection method based on a bipolar-electrode based fluorescence for electroactive compounds separated by ME is presented.

The initial detection system consisted of a simple T microchip as the separation channel and a straight channel (with two reservoirs) as the fluorescent probe flow channel. Both the separation and the flow channels were aligned on the same electrode and was biased externally as a bipolar electrode. The success of the new system was demonstrated in the reductive electrochemical mode using two model analytes. The main challenge of this system is the higher electrochemical background current at the working electrode. This challenge was overcome by introducing a sacrificial electroactive compound at the oxidative end of the bipolar electrode. Better S/N ratios were obtained by modified system compared to amperometric detection. This system will ultimately be applied in the oxidative mode for the detection of nanomolar concentrations of RNOS.

**Keywords:** Biosensors, Electrophoresis, Fluorescence, Method Development

**Application Code:** Bioanalytical

**Methodology Code:** Microfluidics/Lab-on-a-Chip
**Abstract Title**: Voltammetric Determination of Diffusion Coefficients Under Non-Ideal Conditions

**Primary Author**: Bradley P. Hambly  
University of Memphis

**Co-Authors**: Bradford D. Pendley, Erno Lindner

**Abstract Text**

Diffusion-controlled transport of ions and molecules through polymer membranes is often the key factor determining the response of chemical and biosensors. Consequently, a variety of methods have been developed for the determination of diffusion coefficients. Voltammetric methods stand out due to their simplicity and low cost of the analysis. When voltammetric methods, e.g., linear sweep voltammetry, are implemented in aqueous solutions, the experimental conditions can be set in a way that the model assumptions for deriving the response function of voltammetric working electrodes are met and the diffusion coefficients can be calculated from the peak or steady-state currents described by the Randles-Sevcik or microelectrode equations, respectively. However, the diffusion coefficients calculated from non-ideal voltammograms, e.g., from voltammograms recorded in highly resistive, complex matrices, such as plasticized polymeric membranes, can be considerably biased.

In our contribution, we present a generally applicable simple protocol for the rapid determination of diffusion coefficients. The method was developed through simulations of Linear Sweep Voltammograms (LSVs), using a modified version of Nicholson and Shain’s equation, and was validated by the computationally more complex model of Aoki and Osteryoung. No statistically significant difference was found in the scan rate dependence of the peak/steady state current ($i_{peak/SS}$ vs. $v^{1/2}$) when calculated with the modified equation of Nicholson and Shain or the model of Aoki and Osteryoung. Using the proposed protocol, the diffusion coefficients can be estimated with a maximum systemic error of 14% due deviations from the theoretical model assumptions. However, when an approximate value of the diffusion coefficient is available, the experimental conditions can be optimized. By selecting an optimized scan rate and working electrode radius the maximum systemic error can be significantly reduced.

**Keywords**: Electrochemistry, Method Development, Voltammetry

**Application Code**: Biomedical

**Methodology Code**: Electrochemistry
Neurochemical Electrochemistry

Enzyme-Free Glutamate Sensing at Carbon Fiber Microelectrodes

Glutamate is one of the most abundant and important signaling molecules in the brain, present at resting extracellular concentrations up to 30 µM. It is highly desirable to monitor glutamate’s synaptic activity to better define this molecule’s roles in health and disease, but there persist analytical limitations for high time resolution glutamate measurements that would reflect synaptic activity. This is because glutamate is a non-electroactive ion at biological pH and because this messenger has limited geographical scope outside of the synapse during transmission. As a step towards local, high time resolution measurements of glutamate, we introduce an enzyme-free, ionophore grafted carbon fiber microelectrode for fast and selective direct glutamate detection. The design of our novel ionophore incorporates copper ion electrochemistry at the carbon fiber, which changes character upon binding with glutamate, thus facilitating a voltammetric response from a non-electroactive analyte. This method contains the classic hallmarks of fast scan cyclic voltammetry, particularly, the capability to monitor transmitter release and reuptake on neurotransmission time scales, with minimal tissue damage in local areas. The work featured here details the development and characterization of this innovative detection platform for in vivo sensing. This technology has the potential to provide a greater understanding of the mechanism controlling synaptic transmission of glutamate and further decipher glutamate’s role in neurological disorders and neurodegenerative diseases.


Keywords: Chemically Modified Electrodes, Neurochemistry, Sensors, Voltammetry

Application Code: Neurochemistry

Methodology Code: Electrochemistry
Synaptic plasticity is one of the most important and fascinating properties of the mammalian brain. However, the process by which cells initiate this plasticity is not fully known. Synaptic plasticity can be related to individual exocytosis release events. Also, it has been speculated that partial release involves the pore opening just the right amount to release the necessary fraction of neurotransmitter at the necessary rate. This has been shown in simple model cells such as chromaffin cells and PC12. In this study we investigated this phenomena in a complex system model.

In our previous study, we determined the amount of octopamine released from single exocytosis events in live, dissected Drosophila larvae neurons. The largest subset of the events showed a single rise and decay that was named simple events and involved the release of 22,000 molecules. Moreover, different shapes of peaks were distinguished which was related to the mechanism of fusion pore opening and closing. In this study, to determine the total content of neurotransmitter inside the vesicle, we applied intracellular impact electrochemical cytometry inside a single nanometer cell varicosity. A nanotip conical carbon fiber electrode was inserted into the bouton of Drosophila larvae. By applying 900 mV potential (octopamine oxidation potential) vs Ag/AgCl reference electrode, transient signals was observed. The average mole amount of octopamine per vesicle was found to be 442000. By combining these two developed techniques we calculated how many molecules are released per pore opening in complex exocytosis events and vesicle content, which suggests a very small fraction of the vesicular content is released during exocytosis at neuronal varicosities.
Improving Selectivity for Fast Scan Voltammetric Measurements Using a Double Waveform and Partial Least Squares Regression

Hydrogen peroxide (H2O2) is a reactive oxygen species whose concentration can rapidly fluctuate with precise spatial and temporal resolution such that functional levels can be achieved for signaling, or gradually increase over many minutes in instances of oxidative stress. Studies to elucidate these dynamics and the diverse roles that H2O2 plays in complex biological environments have been hindered by the lack of a method for probing H2O2 fluctuations in living systems with molecular specificity. Background-subtracted fast-scan cyclic voltammetry (FSCV) at carbon-fiber ultramicroelectrodes provides a method of detecting H2O2 with high spatiotemporal resolution that can be used in awake and behaving animals. However, H2O2 signals are commonly accompanied by shifts in pH (pH) and electrode drift, which limit the measurement window to approximately 90 seconds. Both interferents hinder quantification of H2O2 dynamics, as the voltammograms for these species can be quite large and can significantly overlap with that for H2O2. We present a method for removing electrode drift and pH contributions from complex voltammetric data. By employing two distinct potential waveforms per scan, one in which H2O2 is electrochemically silent and a second in which electrode drift, pH, and H2O2 all contribute to the signal, a clear distinction between these species is established. A partial least squares regression model permits electrode drift and pH signals to be predicted and subtracted from the complex signal, so as to resolve H2O2 dynamics over extended measurement windows. Models were constructed and validated with [in vitro] analyses, as well as data collected in an awake animal, using the 99.5% cumulative variance and k-fold cross validation methods. This double waveform partial least squares regression model is a powerful tool that can be used to disambiguate and evaluate naturally occurring H2O2 fluctuations, and to extend the measurement window for FSCV investigations.

Abstract Text

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Keywords: Bioanalytical, Data Analysis, Microelectrode, Voltammetry
Application Code: Neurochemistry
Methodology Code: Electrochemistry
The most common \[i\]in vitro[/i\] method of determining enzyme rates is typically an initial rate experiment, in which an enzyme is mixed with a large excess of substrate to reach a quasi-steady-state. In these experiments, there is control over the concentration of enzyme, substrate, and the exact time when the two components are allowed to react. However, for \[i\]in vivo[/i\] monitoring of enzyme activity, things are not so simple. Our lab developed a method for measuring enzyme activity in the extracellular space called electroosmotic push-pull perfusion coupled to capillary liquid chromatography (EOPPP-cLC). In this method, an exogenous substrate can be introduced via a source capillary to the extracellular space of live tissue and any hydrolysis products can be recovered via a second sampling capillary. Any unreacted substrates and formed products can be quantified by cLC. Understanding how one can extract useful information about enzyme rates in intact ECS given how much product and/or substrate is collected can be a challenge. We created a finite element model to not only guide experiments but also guide data analysis to extract this exact information. We found that despite there being a concentration gradient of the substrate and the product as well as a distribution of reaction times in the tissue ECS, we can still fit the Michaelis Menten equation to the perfusion data and obtain $V_{\text{max}}$ and $K_{m}$. Using these simulations as a guide, we determined the $V_{\text{max}}$ and $K_{m}$ of a membrane-bound aminopeptidase that hydrolyzes Leu-enkephalin into GGFL in the intact ECS of hippocampal tissue. We measured a 3-fold higher activity of this enzyme in the CA1 than the CA3 of the rat hippocampus and showed that inhibition of this enzyme is neuroprotective to the CA1 when the hippocampus undergoes ischemia. Furthermore, our values of $V_{\text{max}}$ and $K_{m}$ are comparable to those previously reported using conventional methods.
Carbon nanomaterials have been used to fabricate microelectrodes to improve their sensitivity, electrocatalytic effect, and time response for the electrochemical detection of neurotransmitters. The carbon nanohorn (CNH), a closed cone-shaped cage of \( \text{sp}^2 \)-hybridized carbons, is a promising nanomaterial to improve carbon-fiber microelectrode (CFME) due to its high specific surface area and edge planes. In this study, we used electrodeposition as a simple technique to deposit CNHs on the cylindrical CFME to prepare the CNH-modified CFME (CNH/CFME) for sensitive detection of dopamine. The optimized concentration of CNH is 0.5 mg/mL, and the optimized electrodeposition waveform is 10 cycles of triangular waveform scanned between -1.0 V and +1.0 V. Using fast-scan cyclic voltammetry (FSCV), the optimized CNH/CFME enhances dopamine peak current 2.3 ± 0.2 times that of the unmodified CFME. In addition, we investigated the oxidative etching of CNH/CFME to obtain the oxidized-CNH/CFME (ox-CNH/CFME), which further increases the peak current to 3.5 ± 0.2 times that of the unmodified CFME because more surface oxide groups were generated from the etching. Morphology and distribution of CNH particles on the carbon-fiber surface, limit of dopamine detection, and electrochemical signals from other neurotransmitters at the modified electrodes were studied. Stability and biofouling properties of the modified electrodes will be also determined to demonstrate their potential for neurochemical studies. These CNH-modified microelectrodes are advantageous for dopamine detection because of their easy fabrication and improved sensitivity.

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**Keywords:** Electrochemistry, Microelectrode, Neurochemistry, Voltammetry

**Application Code:** Neurochemistry

**Methodology Code:** Electrochemistry
Neurochemical Electrochemistry

Detection of Chemically Stimulated Dopamine Release in Adult \textit{Drosophila melanogaster} Brain

Dopamine is an important neurotransmitter in the central nervous system influencing various behavior outputs and involved in neurodegenerative diseases. Previously, our group established methods to measure chemically and optogenetically stimulated dopamine release in \textit{Drosophila} larvae brains using fast-scan cyclic voltammetry (FSCV) at a carbon fiber microelectrode. In this study, acetylcholine, a major excitatory neurotransmitters in \textit{Drosophila}, was used to evoke dopamine release in harvested adult brains. Our result indicated that evoked dopamine release in male is not significantly different than that of female at age of 4 to 10 days old. Moreover, treatment with 3-iodo-L-tyrosine (3-IT), an inhibitor of tyrosine hydroxylase, diminished dopamine release and no changes in stimulated release were observed in octopamine synthesis knock out flies. These results indicated that observed signal is due to dopamine release. Treatment with tetrodotoxin, a sodium channel blocker, and alpha-bungarotoxin, a nicotine acetylcholine receptor antagonist, resulted in decrease stimulated dopamine release. Dopamine release and uptake were also studied in \textit{fumin} dopamine transporter mutant flies (\textit{fmn}). In addition, stimulated release in \textit{fmin} and control flies fed with methamphetamine (METH) was measured and compared at 24 hrs or 96 hrs. We observed that dopamine release was significantly increased in control flies fed with METH but no changes observed in \textit{fmin} flies. Collectively, these results demonstrated that acetylcholine stimulated dopamine release in the intact adult brain is an essential step for carrying out further studies in \textit{Drosophila}.

Abstract Text

Bioanalytical, Electrochemistry, Method Development, Neurochemistry

Application Code: Neurochemistry

Methodology Code: Electrochemistry
Adenosine is a neuroprotective endogenous agent that modulates important physiological process in the central nervous system. Spontaneous, transient adenosine has been recently identified as a rapid mode of signaling that lasts about three seconds. However, whether this transient adenosine release is different between mouse brain regions is not fully known. Here, the number, concentration, and duration of spontaneous adenosine events in the prefrontal cortex, hippocampus and caudate putamen of anesthetized mice were characterized and compared. The number of adenosine release events in prefrontal cortex was significantly lower (averaging 37 +/- 7 per hour) than caudate putamen (averaging 54 +/- 9 per hour) and hippocampus (averaging 52 +/- 10 per hour). However, the average concentration and duration of adenosine in the prefrontal cortex (0.24 +/- 0.04 [micro]M and 1.8 +/- 0.1 s), caudate putamen (0.31 +/- 0.03 [micro]M and 1.9 +/- 0.1 s) and hippocampus (0.27 +/- 0.03 [micro]M and 1.7 +/- 0.1 s) were not significantly different. As a result, spontaneous adenosine signaling differs in the number of transients, indicating that the spontaneous adenosine activating receptors as neuromodulators may be controlled by the events numbers.
The uniformity of the active pharmaceutical ingredients (APIs) in a batch of solid dose pharmaceuticals is defined by the US Pharmacopeia in terms of API content uniformity or weight variation for the batch. The former applies to tablets containing API doses less than 25 mg or 25% of the tablet weight. For the past 30 years, high performance liquid chromatography (HPLC) has been the method of choice to determine the average concentration for a set of tablets and if the batch is within pre-defined limits. For example, 10 tablets are dissolved in the appropriate solvent and then measured. In the past decade, Raman spectroscopy has been investigated as a simpler, faster method to qualify batches of solid dose pharmaceuticals. Raman spectra can be obtained by simply directing a laser into a sample, collecting the spectrum, and quantifying the API by applying traditional univariate (e.g. peak height), or multivariate calibration model. The entire measurement time is often less than 1 minute. In this case, however, 10 individual tablets are measured to determine the average concentration and standard deviation. However, to our knowledge, no one performed a 1-to-1 comparison of individual tablet API concentrations measured by both HPLC and Raman. Here we present such a comparison for Claritin tablets.
Multimode chromatography is gaining increasingly attention in recent years, owing to better understanding of multimode interactions and commercialization of modern multimode columns. While reversed-phase columns (e.g. C18) are commonly used for a broad range of applications, they often fail to retain hydrophilic analytes, and offer limited selectivity. Multimode chromatography provides a viable solution to such challenges. Multimode chromatography is a chromatographic technique in which solutes interact with stationary phase through more than one interaction mode. Due to the multiple retention modes that a single multimode column can offer, it often provides additional dimension to a separation method by adjusting the mobile phase conditions, resulting in superior applications in the separation of compounds that are not retained or not well resolved by typical reversed-phase LC methods, especially for polar and charged molecules. A variety of applications such as pharmaceutical counter ions and drug substances, glycans, peptide mapping, surfactants and pesticides, have been demonstrated on general multimode chemistry platform, however, very often column architecture or chromatography conditions need to be tailored to address specific applications. This presentation will discuss multimode stationary phase architecture, including application-driven column chemistry design, synthetic strategy and method development. In addition to an overview of modern multimode column technology, chemistry design strategies for pharmaceuticals, biopharmaceuticals and proteomics applications will be elaborated.

Keywords: Biopharmaceutical, Consumer Products, HPLC Columns, Pharmaceutical
Application Code: Pharmaceutical
Methodology Code: Liquid Chromatography
Chiral liquid chromatography is a very important aspect in the quality control of pharmaceuticals, agrochemicals, consumer products, among others. Surprisingly, before 1992, The Food and Drug Administration did not have any regulations pertaining chiral compounds. Today’s drug industry must provide identification and characterization of each individual isomer of a new racemic mixture. Chiral liquid chromatography has been governed as early as 1980’s by stationary phases composed of macrocyclic glycopeptides (vancomycin and teicoplanin), cyclic oligosaccharides (cyclodextrin and cyclofructan), and polysaccharides (cellulose and amylose). The derivatization of such structures and their mechanism of selectivity have been studied throughout the years. New advances of silica manufacturing as the common solid support have allowed commercially available LC columns to transition from the standard 5 µm Fully Porous Particle (FPP) to 2.7 µm Superficially Porous Particle (SPP). Chiral SPP columns have shown higher efficiencies at high flow rates, resulting in ultrafast, sub-minute enantiomeric separations. In recent studies, synthetic modifications of macrocyclic glycopeptides have shown complementary selectivity compared to their natural product. Two macrocyclic glycopeptide analogs have been synthesized, covalently immobilized to SPP, and their chiral recognition assayed against a library of commercially available racemic drugs. Among the compounds of interest, one of the chiral phase analog have shown unique selectivity towards nicotine. To our knowledge, this is the first time that nicotine enantiomers can be resolved with such selectivity and analysis time. Currently, the further application of these two new phases and their retention mechanism are being evaluated. The advance of solid supports along with the development of novel stationary phases will aid in the fast and efficient analysis of many commercialized racemic chemicals used worldwide.
Clorsulon is used for the treatment and control of liver flukes (Fasciola hepatica) in livestock. A reversed phase high performance liquid chromatography (RP-HPLC) method has been developed for the identification and assay of clorsulon and estimation of its degradation products in a Clorsulon Injectable product. Clorsulon injectable is dissolved in a diluent composed of 65% Water (H2O)/ 35% Acetonitrile (ACN) (v/v) to a target concentration of 0.2 mg/mL. Analytes are separated on ACE EXCEL SUPER C18 (50 mm x 4.6 mm i.d., 3 µm particle size) maintained at 35 °C in an HPLC system with a gradient elution using mobile phases containing 0.05% phosphoric acid (H3PO4) in water as mobile phase A and Acetonitrile (ACN) as mobile phase B and detected with UV detection at 267 nm. The run time is 11 minutes. Clorsulon is identified by retention time match and quantitated against an external clorsulon standard. Clorsulon degradation products are identified by relative retention time match and quantitated against clorsulon. The quantitation limit is 0.10% of the target sample concentration. The method was fully validated and has been demonstrated to be accurate, robust, specific, and stability indicating. This method is suitable for routine release and stability testing of the Clorsulon in the Clorsulon Injectable product in a QC laboratory.
The predominant enantiomer of nicotine found in nature is (S)-(nicotine), and its' pharmacology has been widely established. However, pharmacologic information concerning individual enantiomers of nicotine and nicotine derivatives are limited. Fast and mass spectrometry compatible methods for the enantioseparation of many nicotine related compounds have not been established using liquid chromatography, and might become more useful as the FDA investigates the need for future regulation of tobacco products. Macrocyclic glycopeptides are known to provide high resolution for a variety of acidic, basic, and neutral compounds by their diverse interactions. However, these antibiotics have not been able to resolve nicotine enantiomers until recently, with a modified macrocyclic glycopeptide. This stationary phase was utilized as a chiral selector bonded to core-shell particles, also called superficially porous particles. The separation of nicotine with this phase was recently reported, and to continue the investigation of this stationary phase a similar methodology was applied to other nicotine related compounds. These compounds included tobacco alkaloids, nicotine metabolites, tobacco-specific nitrosamines, and synthetic nicotine derivatives. Most compounds were easily baseline resolved with the modified macrocyclic glycopeptide by HPLC and proven to be mass spectrometry compatible. The modified macrocyclic glycopeptide has shown complementary separations with other macrocyclic glycopeptides, which resolved most of the other nicotine related compounds. Interestingly, the tobacco-specific nitrosamines were not easily separated by these macrocyclic glycopeptides, in part because they were discovered to form E/Z stereoisomers. Therefore, other stationary phases, such as cyclodextrins and quinine based chemistries were applied. This methodology could lead to future pharmacological studies and details in HPLC parameters will also be discussed.

Keywords: Chiral, Chromatography, HPLC Columns, Toxicology
Application Code: Pharmaceutical
Methodology Code: Liquid Chromatography
Pharmacetical - Liquid Chromatography

Development of a Multi-Step Synthetic Peptide Purification Process

Peptide synthesis technology has developed sufficiently to allow for large scale manufacturing of peptides. The Pharmaceutical Industry is extremely interested in using peptides as therapeutic agents and has been a significant driver of this technology. Isolation and purification of the desired peptide product from the crude synthetic mixture is a key aspect of this technology and has also been developing along with the synthesis technology. A multi-step purification process is often employed for isolating and purifying crude synthetic peptide mixtures. The first step is designed to isolate the desired material by removing most of the undesired components. This step is often but not always a chromatographic step. The isolated material is then purified further by one or more different chromatographic steps to “polish” the material to the desired purity level. These different steps are typically complimentary forms of chromatography such as ion exchange, gel permeation, affinity, and reverse phase.

This work follows a two-step purification concept where both steps are accomplished utilizing the same stationary phase. The example presented for this strategy is the purification of a crude sample of Liraglutide, a commercially significant synthetic peptide. The two steps have different selectivity based on changes in mobile phase pH and choice of organic solvent. Our data follows the development of this purification process from the initial crude sample to the isolation of the final product. Detailed results for the purity and yield for each step are presented.

Keywords: Peptides, Pharmaceutical, Prep Chromatography
Application Code: Pharmaceutical
Methodology Code: Liquid Chromatography
Pyriproxyfen is a juvenile hormone analog and an insect growth regulator. It prevents larvae from developing into adulthood and thus rendering them unable to reproduce. Therefore, it was used as an active pharmaceutical ingredient (API) in animal health products for flea control in dogs and cats. Based on the knowledge of the authors through literature search, there is no published stability-indicating HPLC method for Pyriproxyfen. A stability-indicating reversed-phase HPLC-UV method was developed for the assay of Pyriproxyfen and estimation of its related compounds in Pyriproxyfen API. Chromatographic separation of Pyriproxyfen and its related compounds was achieved by using an gradient elution at a flow rate of 1.0 mL/minute using 5 mM ammonium acetate in water as mobile phase A and acetonitrile as mobile phase B. Analytes are separated and quantitated by an external calibration against Pyriproxyfen standard solution at 275 nm using ACE Excel Super C18 column (4.6 mm I.D x 50 mm length, 3.0 µm particle size) as the primary column and YMC-Triact C18 (50x4.6mm, 3.0µm particle size) as the equivalent column at 40°C. The total run time for this method is 15 minutes. This method was successfully validated according to International Conference on Harmonization (ICH) guidelines and was found to be specific, linear, accurate, precise, robust and sensitive. The stability indicating capability of the method was demonstrated through adequate separation of all potential Pyriproxyfen related compounds (from Pyriproxyfen and from each other) that are present in stressed samples under heat, light, base, acid and oxidation. This reversed phase HPLC-UV method is suitable for routine release and stability test for Pyriproxyfen API in QC laboratories.

Keywords: HPLC, Method Development, Pharmaceutical, Validation
Application Code: Pharmaceutical
Methodology Code: Liquid Chromatography
During shelf-life determinations, whether in real time or by accelerated exposures, error bars in measured degradant levels define the confidence intervals for extrapolations, both for extended times and from high to low temperature/relative humidity. The precision plays an especially important role when these extrapolations are very large resulting in significant divergence in the prediction intervals. The present investigation used test drug products to distinguish three categories of error sources: (1) dosages themselves, (2) extraction and sample preparation, and (3) HPLC peak integration. These error source contributions were examined at different degradant levels generated by elevated temperature exposures. The experiment involved quantifying errors (standard deviations) from multiple dosages (tablets), from preparations of composite tablets and from multiple injections of prepared samples. From these analyses, it was possible to determine under what scenario each error source becomes dominant. Based on these results, recommendations are made for improving precision in degradant level measurements.

Keywords: Liquid Chromatography, Pharmaceutical, Quantitative, Statistical Data Analysis
Application Code: Pharmaceutical
Methodology Code: Liquid Chromatography
Surface enhanced Raman spectroscopy (SERS) is widely reported as a possible tool for trace chemical detection. Planar SERS substrates are formed when nano-geometrical structures coated with a metal film. The combination of geometry and metal type provides for plasmonic enhancement with a particular laser excitation wavelength. While the mechanisms governing the SERS response are well understood (chemical and electromagnetic enhancement), much of the attention has been focused on factors to improve the electromagnetic enhancement. However, relatively little work has been done on probing the influence of thermodynamics of metal-molecule interactions on SERS response, even though binding is essential for a SERS spectrum to be observed. Molecular interactions between the analyte and SERS substrate metal drive the formation of self-assembled molecular monolayers (SAM). These molecular interactions are strongly dependent on types of metal, geometries of the SERS substrate, molecular parameters (such as dipole moment) of the binding molecule and solute-solvent-metal interactions. To probe these influences, we studied the formation of mono-molecular layers for several nitrogen and sulfur containing aromatic chemicals on planar SERS substrates of varying geometries and noble metal layers. Solvent-solute-metal inactions were also studied with ethanol (polar protic), acetonitrile (polar aprotic), water (polar), and dodecane (non-polar) as solvents. Our studies reveal the combined influence of metals, geometries, and solvation on the effectiveness of planar SERS substrates as a tool for trace detection.
The ability to detect biomarkers of Parkinson’s disease (PD) in biofluids requires the development of new sensing technologies that are rapid, label-free, require little processing of the sample, detect multiple targets simultaneously, and are stable over relevant timescales. The majority of existing detection methods require multiple steps to process complex biological samples. Raman spectroscopy (RS) provides excellent chemical specificity with simple instrumentation and requires little to no sample processing. There is also no interference from water in RS as seen in several other techniques. The disadvantage is the inherently weak signal associated with Raman spectroscopy. Using enhancement techniques such as surface enhanced Raman spectroscopy (SERS) or resonance Raman spectroscopy greatly increase the Raman signal and allow detection of very low concentration analytes. We aim to characterize the vibrational signatures of dopamine and its metabolites in order to establish a biomarker for PD.

Keywords: Raman Spectroscopy, Surface Enhanced Raman Spectroscopy
Application Code: Neurochemistry
Methodology Code: Vibrational Spectroscopy
Nitramine compounds are used in weapon systems as constituents in every formulation and as the secondary explosives in largest amounts. Here, a novel nano-analytical method based on surface-enhanced Raman spectroscopy (SERS) was proposed for the first time for simultaneous quantification of nitrite (NO$_2^-$) obtained from octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX) and hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX). Beside identification of explosives with Raman spectroscopy, it is important to quantify them in real samples with a low detection limit. For this purpose, the nitrite derivation for RDX and HMX was performed at 60°C with alkaline hydrolysis; however only RDX was hydrolyzed at room temperature. Due to the fact that HMX and RDX show different hydrolysis behavior, these nitramines could detect separately and each of them could quantify. SERS spectra were obtained on the surface of 4-aminothiol modified (4-ATP) gold nanorods (AuNPs) doped with silver nanoparticles in the presence of N-(1-naphthyl)-ethylenediamine (NED). The maximum intensity band was observed at 1286 cm$^{-1}$ and the correlation was found to be linear within the standard solution concentration range of 0.25 – 10 mg/L. In addition, the recovery of the method was higher than 90%, and by using SERS, it was shown that the limit of detection was found to be lower than 1 mg/L. Moreover, the proposed method was validated against 4-ATP-AuNPs+NED colorimetric method and it proved to be more sensitive and accurate than existing colorimetric techniques. This new method is applicable to commercial grade RDX sample for detection of HMX as an impurity and to soil samples containing explosive residues.
Surface Enhanced Raman Selective Analysis of Cell Organelles by Surface-Enhanced Raman Scattering Spectroscopy

As the vital organelles of cells, e.g. nucleus, mitochondria and lysosome, can participate in many important physiological and pathological processes including cell proliferation, organism metabolism and intracellular transportation and play important roles in regulating cellular biological functions. Raman spectroscopy as one of promising fingerprint technique has been widely used for analysis of multiscale biomolecules from small amino acids to DNAs, peptides, proteins, as well as tissues and tumors, due to its notable superiority in the analysis of multi-component detections and less interference from water. Intrinsic components of a cell provide so weak Raman signals that they require high laser power and long collection time for an identifiable spectrum, but causing photo damage on cells. Also, the Raman signals involving all components are difficult to analyse. Here, with the aid of peptide functionalized Au nanorods (AuNRs) having high signal amplification capability and organelle targeting function, high-quality surface-enhanced Raman scattering (SERS) spectra of the specific organelles adjacent to AuNRs were selectively obtained. The intrinsic components of cell organelles were analysed and compared at the molecular level, which reflects more information of cells themselves relative to most of studies in which the encoded SERS tags were adopted in cell-involved systems and only the encoded molecules were traceable. This study is of great significance in explorations on organelles and their microenvironments during physiological, disease progression and treatment processes from subcellular level.

Keywords: Bioanalytical, Biospectroscopy, Surface Enhanced Raman Spectroscopy
Application Code: Bioanalytical
Methodology Code: Vibrational Spectroscopy
The catalytic properties of plasmonic nanomaterials are increasingly used to address major global problems, such as generating renewable fuels and reducing carbon dioxide levels. The production of hot electrons from the absorption of visible light by plasmon resonances provides efficient light collection across the entire electromagnetic spectrum. Using nanomaterials alone, or coupling with semiconductors in hybrid structures, improves catalytic efficiencies as much as 4-fold over simple semiconductors. These improvements are not sufficient enough, however, to compete with traditional methods (fossil fuels) in cost or efficiency. Plasmon catalytic efficiencies must be improved greatly to make up for the high costs of noble metals. Improving surface reactivity (electron-hole pair separation) may be the answer, but this requires better understanding of the mechanisms controlling photocatalytic reactivity.

This research aims to gain a greater understanding of local surface potentials that affect the reactivity of plasmonic nanomaterials using the vibrational Stark effect. Results show a surface charge is created on nanostructured materials arising from the second order nonlinear process of optical rectification. By adsorbing an electric field reporter molecule (4-mercaptobenzonitrile) onto the plasmonic materials, the surface charge can be monitored by changes in the nitrile stretching frequency. Using a photocatalytically active molecule, either 4-nitrothiophenol or 4-aminothiophenol, the reduction or oxidation to 4,4-dimercaptoazobenzene can be monitored on a gold nanorod surface. Through Raman spectro-electrochemical methods, it was determined that plasmonic reactivity is dependent on local surface potentials, and therefore influenced the optically rectified electric field. This finding suggests that improving the properties of plasmonic materials, which give rise to this second order phenomena, could lead to improved catalysts for commercial use in the future.
Surface Enhanced Raman
SERS-Based Immunobiosensor for Bacteria Detection

Surface-enhanced Raman scattering (SERS) is a powerful technique for detection and identification of biological structures. SERS-based immunoassay methods are mostly used for the specific detection and identification of bacteria. In this study, a novel SERS-based immunobiosensor for detection of Methicillin-resistant Staphylococcus aureus (MRSA) is developed. First, SERS substrate is prepared by deposition of concentrated spherical silver nanoparticles (AgNPs) on a regular glass slides with convective assembly method. Second, the surface is modified with 4-Aminothiophenol (4-ATP). 4-ATP is used not only to bind the antibody for MRSA to the surface but also to monitor peak shift. Then the antibody is bound to the 4-ATP modified surface through amide bond. Finally, the modified surface is incubated with the bacteria and obtained SERS spectra from the surfaces. The peak shift at certain peaks on 4-ATP molecule is monitored to detect the bacteria. The results demonstrate that, MRSA can be detected using SERS-based immunobiosensor based on the peak shifts.

Bioanalytical, Biological Samples, Biosensors, Surface Enhanced Raman Spectroscopy

Bioanalytical
Vibrational Spectroscopy
We have developed a simple and rapid method for the accurate subtyping of avian influenza viruses (AIVs) using modified gold nanoparticles as labels and surface-enhanced Raman spectroscopy (SERS) for detection. Anti-H3 AIV antibodies and a Raman reporter molecule were co-immobilized onto gold nanoparticles. The functionalized gold nanoparticles were mixed with sample, and H3 AIVs mediated the aggregation of the nanoparticles. Aggregates were captured and concentrated on a polycarbonate track-etched membrane filter and analyzed via SERS. In this work we screened and identified a polyclonal antibody with broad intra-subtypic affinity for H3 AIVs and minimal affinity for other AIV hemagglutinin subtypes. Additionally, we developed a novel approach to chemically modify the antibody to facilitate irreversible adsorption onto the gold nanoparticles to form stable conjugates in the biological matrix, i.e., allantoic fluid. The optimized SERS assay required 60 minutes from sampling to analysis and resulted in a 20-fold lower detection limit than ELISA. This assay was employed for the analysis of seven AIVs isolated from shorebirds and correctly identified all H3 isolates with no false-positive results. The potential advantages of this platform relative to existing technologies was demonstrated, and future efforts will focus on multiplexed detection using spectroscopically and immunologically unique SERS labels to realize the full potential of SERS-based detection.

Keywords: Bioanalytical, Immunoassay, Surface Enhanced Raman Spectroscopy
Application Code: Bioanalytical
Methodology Code: Vibrational Spectroscopy
Integrin receptors are an integral feature of a cell membrane and play an important role in regulating cellular activities such as cellular migration, invasion and proliferation in tumours. They have a well-known affinity for small peptides that contain arginine-glycine-asparate (RGD). Therefore, by targeting integrin receptors present in cancer cells with specific RGD containing ligands, the ligand-receptor binding chemistry can be investigated and provide an insight which could be key to cancer prognosis and treatment. Gold nanostars are highly attractive for biological application, such as integrin targeting, which can be achieved by utilising their surface chemistry, facile synthesis and optical properties. We therefore exploit the intense surface enhanced Raman scattering (SERS) and tuneable plasmon resonance properties of gold nanostars functionalised with cyclic RGD ligands for the detection of \( v_3 \) integrin on a cell membrane. Gold nanostars were synthesised using a variety of different methods such as gold/silver seed and seedless approaches and their SERS response investigated using 4-mercaptobenzonitrile (4-MBN). The nanostars which achieved the biggest SERS enhancement of 4-MBN were then functionalised with the ligand cyclic-RDGFC and the SERS response from their interaction with \( v_3 \) integrin are reported from isolated proteins and intact cancer cell membranes. These results demonstrate a label free approach investigating the binding of small molecules ligand in intact cell membrane.
Background: Computer Aided Drug Design (CADD) molecular docking program used in this study and as a result Rifabutin was retrieved as structurally similar drug molecule to lixisenatide with significant binding efficacy towards glucagon like peptide-1 receptor (GLP-1R). To afford beneficial reason in-vitro study conducted which too provides a beneficial mark for prospective inhibition of α-glucosidase and α-amylase enzyme and thereby evaluated for its antidiabetic potential against streptozotocin (STZ) and nicotinamide (NA) induced diabetes in albino wistar rats.

Result: Among different concentrations of Rifabutin employed, the lowest (20 mg/kg), showed highest inhibition against α-amylase (89.3%) and α-glucosidase (73.3%) in-vitro in a dose-dependent manner; an outcome found to be about 20% higher than Acarbose (53%), used as a standard α-glucosidase inhibitor. Rifabutin demonstrated significant efficacy towards restoring the blood glucose, insulin and distorted lipid profile in the animals treated with STZ-NA. Rifabutin also regulated the inflammatory (Nitric Oxide) and oxidative stress (TBARs, SOD, Catalase, GSH and protein carbonyl) markers in the pancreatic tissue of the STZ-NA treated animals. When scrutinized on the grounds electrocardiographic and heart rate variability (HRV) changes, Rifabutin decreased the heart rate, without much affecting the QRS, QT and QTc interval. In the same line, HRV analysis revealed increase in the LF/HF suggesting sympathetic predominance in the diabetic animals, which was very well restored by the Rifabutin. Rifabutin was also evident to be less hepatotoxic in comparison to the standard metformin, providing an added advantage as an anti-diabetic agent.

Conclusion: The study concluded that the low dose of rifabutin favourably regulate of the blood glucose and other biochemical paradigms in comparison to high dose of rifabutin.

Keywords: Drug Discovery, Natural Products, Pharmaceutical, Toxicology
The newly synthesized two series namely: (Z)-N-(2-(benzo[d]thiazol-2-ylthio)acetyl)-2-benzylidenehydrazinecarboxamide / (Z)-2-(benzo[d]thiazol-2-ylthio)-N-(2-benzylidenehydrazine carbonothioyl)acetamide (3a-3r) were prepared. These compounds synthesized from 2-mercaptoBenzothiazole(MBT), Thiosemicarbazide (TSC) and Semicarbazide (SC) were crucial functionalities containing the wide variety of biological activities and have a broad range of therapeutic properties. The structure of the synthesized compounds was confirmed by spectral data and evaluated for their in vitro antibacterial activities against Gram-positive and Gram-negative bacteria.
The current study was to examine the nephroprotective effect of wedelolactone (WEL) in streptozotocin (STZ)-induced type II diabetic mellitus in Wistar rats. Diabetic nephropathy (DN), is considered as the global health problem, which is commonly occurred during the diabetes as a part of its complications. Several studies showed that current conventional therapies of diabetes using blood glucose-lowering medications have restrictions in avoidance of DN. This has led to increasing consideration of complementary and alternative medicine from natural products having renal protective effect in diabetes with fewer side effects. WEL was isolated by column chromatography method from Wedelia Calendulacea plant and characterized by spectroscopy. The Swiss albino wistar rats were divided into different group and STZ was used to induce the diabetes. The rats were treated with the WEL for 8 weeks post induction of type II diabetes. The body weight, blood glucose level, different biochemical and renal parameters estimated, respectively. Proinflammatory cytokines including interleukin-1β (IL-1β) and interleukin-6 (IL-6), inflammatory mediators were also estimated. Western blotting was used for the estimation of renal NF-κB, TNF-α, TGF-β, collagen-IV and fibronectin level, respectively. The result clearly suggested that WEL significantly (p<0.001) abrogated inflammation, oxidative stress and renal dysfunction at dose dependent manner. WEL significantly up-regulated the plasma insulin and down-regulation of blood glucose. Additionally, WEL suppressed the TNF-α expression and NF-κB activation. Moreover, WEL altered the pro-inflammatory cytokines and down-regulated the collagen-IV, fibronectin, up-regulated the renal pathology and decreased the expression of collagen-IV, fibronectin, TGF-β in renal tissue and improved the renal pathological condition. Current findings suggested that WEL can prevent the expansion of DN in type II diabetic rats via targeting the multiple pathways.
The cytochrome P450 substrate-inhibitor panel has been designed for preclinical evaluation of drugs’ in vitro biotransformation on a 3D human hystotypical cell model using “liver-on-a-chip” technology. Based on the data reported in the literature, the following specific substrate-inhibitor pairs have been selected: bupropion/2-phenyl-2-(1-piperidinyl)propane (BPP) to evaluate CYP2B6 activity, tolbutamide/sulfaphenazole for CYP2C9, omeprazole/(+)-N-benzylnirvanol (NBN) for CYP2C19, testosterone/ketoconazole for CYP3A4. The concentrations of substrates and inhibitors have been optimized to ensure reliable detection of major metabolites by means of HPLC-mass-spectroscopy. The specificity of the developed panel for the desired cytochrome P450 isoforms has been demonstrated. The experimental evaluation of the developed protocol has been performed by testing two model drugs, warfarin and dasatinib. The results obtained correspond to the reported mechanisms of both drugs in vitro biotransformation in a “liver-on-a-chip”: biotransformation of dasatinib is catalyzed by CYP3A4, and that of warfarin by CYP2C9. An existing possibility for dasatinib and warfarin interaction with drugs metabolized CYP2C19 isoform is demonstrated. The developed and validated panel has been used to study HIF prolyl hydroxylase inhibitor under development – adaptaquin – and its optimized variants. The optimized adaptaquin variants show no toxicity up to a 100-fold increased range over EC50. The drugs are metabolized by CYP3A4 and CYP2B6. Activation of the latter by the drugs of this groups has been observed, meaning that the drugs under development will increase toxicity of Tylenol.

The work is supported by Russian Scientific Foundation grant 16-14-10226.
Promoter-based luciferase reporters are widely used for research and drug discovery purposes. However, they are slow responding and represent an integral signal that cannot be ascribed to an individual step in activation of a transcription factor. Luciferase fusion reporters of transcription factors regulated by ubiquitinylation and proteasomal degradation such as HIF1 ODD-luc and Neh2-luc reporters exhibit a number of advantages over the common promoter reporters, both in terms of research and drug discovery purposes. The advantages are (1) immediate response to stabilizers of a transcription factor; (2) an order higher absolute luminescence signal than that for the promoter-based reporter; (3) specificity for the drugs working at the rate-limiting step of the reporter performance, (4) selection of cell-permeable and non-toxic drugs under physiological conditions. The new development of such reporters includes a combination of interacting pairs on one and the same reporter vector. This new strategy is illustrated by the construction and characterization of reporters combining HIF ODD-luc and HIF prolyl hydroxylase isoforms (HIF PHDs) for the purposes of screening and optimization of HIF PHD isoform-specific inhibitors. In this case the cell is used as a micro-reactor for an enzymatic assay. It is especially important in the case of low-level expressed enzymes, such HIF PHD3 isoform. This new approach has been used to optimize the structure of branched tail hydroxyquinoline pan-inhibitor of HIF PHDs (adaptaquin) for increasing its preference for HIF PHD1 and HIF PHD3 isoforms over HIF PHD2 isoform.

The work is supported by Russian Scientific Foundation grant #16-14-10226.
A 3D human hystotypical cell model, “brain-on-a-chip”, has been created using differentiated neuroblasto-ma cell line. The neuroblasto-ma cell line has been successfully used in cell-based reporter assays for drug screening purposes, in particular, novel generation of luciferase fusion reporters stably expressed in neuroblasto-ma cell line - HIF ODD-luc and Neh2-luc reporters – easily permit identification of novel molecules stabilizing the corresponding transcription factors - HIF and Nrf2 – and thus inducing antihypoxic and antioxidant genetic programs necessary for cell survival under conditions of chronic and acute neurodegeneration. However, luciferase assay requires lysis of the cell line, and thus, it not applicable for continuous monitoring of reporter activation in the intact cells. Changing firefly luciferase for fluorescent labels such as green and red fluorescent proteins permits direct and simultaneous imaging of the genetic programs triggering in “brain-on-a-chip”. Neuroblasto-ma cell lines transformed with the new reporter constructs have been generated and mixed to be used in the corresponding “brain-on-a-chip”. Novel microfluidic chip has been developed and was made of PDMS/glass using soft lithography technique. The chips can be organized in special adapter for combability with common microplate fluorometers allowing real-time imaging and fluorescence measurement. The approach upgrades the technology to the new level, and is applicable for monitoring drug effects in the continuous regime.

The work is supported by Foundation for Assistance to Small Innovative Enterprises grant #24465.

Keywords: Drug Discovery, Fluorescence, Lab-on-a-Chip/Microfluidics
Application Code: Drug Discovery
Methodology Code: Microfluidics/Lab-on-a-Chip
Cocaine is an addictive stimulant drug, which directly affects the Central Nervous System (CNS). Nowadays, this drug is one of the most used illicit drug in the entire world [sup][1]/sup and it is frequently commercialized containing some cutting agents, pharmacologic substances whose properties resemble the cocaine in order to mimic the pharmacological effect of the illicit drug [sup][2]/sup. Hence, the chemical characterization of cocaine is important to provide chemical and physical information to assist police agencies identifying the drug origin. In this context, the present work aims the use of Boron-Doped Diamond (BDD) electrode for extracting voltammetric information about three cutting agents commonly added to cocaine (Phenacetin, Caffeine and Benzocaine) in order to quantify and classify these substances in real samples using supervised and non-supervised pattern recognition tools, respectively. Good linear correlation between the voltammetric information and the concentration values for two different species in a mixture, phenacetin and caffeine, using multivariate calibration with Partial Least Squares (PLS) were obtained. The PLS model showed a high coefficient of correlation (R[sup]2[/sup] = 0.98) and a satisfactory prediction for unknown samples inside the studied range (0.01 to 0.1 mmol L-1). Additionally, some good correlation with the third compound is already observed too.

Financial support: FAPESP, CNPq and CAPES.
### Abstract Text

A rapid, plate-based photometric method has been developed for assay and degradation product determination in tablets of a drug product. The method will be characterized in terms of sensitivity, specificity, linearity, accuracy and precision. The feasibility of implementing this method at commercialization sites as well as alternative approaches to enable real-time release testing (RTRT) of drug products will be discussed.

### Keywords
- Fluorescence
- High Throughput Chemical Analysis
- Liquid Chromatography
- Pharmaceutical

### Application Code
- Pharmaceutical

### Methodology Code
- Molecular Spectroscopy
The widespread diseases prevailing in the society and the high resistivity of the microbes against the drugs available in the market demonstrates a challenge for the scientific persons working in the field of medicinal chemistry to design and develop novel and effective bio-active molecules. In context to the above issue, the author(s) in the current chapter delivers various methods of synthesis for 1,3,4-oxadizole ring bearing molecules demonstrating extensive biological properties along with an extensive review on current drugs available with the same oxadiazole skeleton and its mode of action. The reason behind selecting 1,3,4-oxadiazole derivatives, is its high versatility in exhibiting excellent potency against several ineffective strains as reported by many researchers. This chapter deals in detail with the review encompassing several illustrations promoting the synthesis of derivatives bearing 1,3,4-oxadiazoles as well as the extent of its various biological properties. Second important factor covered in this chapter is the details of the drugs bearing 1,3,4-oxadiazole nuclei and its medicinal efficacy. A detailed reviews and results on the computational studies including QSAR and docking studies results have been displayed in the chapter. Overall the details provided in the chapter will help the readers to deal with the basic feature of 1,3,4-oxadiazole as well as its chemical reactivity and biological activity.

**Keywords:** Drugs  
**Application Code:** Drug Discovery  
**Methodology Code:** Chemical Methods
Sirtuin-5 (SIRT5), a mitochondrial desuccinylase, has been identified as a possible target for the treatment of metastatic melanomas. As such, there is a pharmaceutical interest in identifying small molecule modulators of SIRT5. Although previous optical, FRET, and HPLC-MS assays have been developed these have been limited by artifactual results or limited throughput for screening. We have previously developed a novel assay in which the peptide substrate and product can be directly visualized with laser-induced fluorescence (LIF) detection and rapidly separated using capillary electrophoresis (CE), in order to assess SIRT5 activity. The goal of this work is to develop a microchip CE (MCE) assay coupled to droplet-based sample introduction with online reagent addition on the nanoliter scale. Using microfluidic chips produced in house and an LIF-detection system, we can achieve separations in 200 ms at a field strength of 3400 V/cm and separation distance of 1 mm. We can also generate nanoliter sized droplets using a CNC milling machine. This assay has been validated using dose-response analysis of the SIRT5 inhibitor Suramin and comparison to literature values.

We are also using ion mobility-mass spectrometry (IM-MS) to elucidate the binding location and stoichiometry of SIRT5 inhibitors. IM-MS and collision induced unfolding (CIU) measurements have been previously shown to be capable of distinguishing differences in the gas phase stabilities of proteins upon ligand binding, and linking those observations to the native protein structure. This fingerprinting approach is also sensitive to the binding mode of a ligand to a protein target. Using a quadrupole-ion mobility-time-of-flight mass spectrometer we were able to see significant differences between apoSIRT5 and inhibitor-bound SIRT5. Current work is focused on building a library of fingerprints using inhibitors with known binding modes.

This work was supported by NIH Grant #R01GM102236
Liposomes have been used in drug discovery and drug delivery for some time, and the biophysical characterization of these systems and their payloads is critical to understanding and optimizing their fabrication and function. This study looks at optimal conditions for extruding liposomes as well as their stability under different conditions. We highlight the limit of detection for fluorescently labeled liposomes. Our aim is to further educate the public about the intricacies of liposome formation and characterization as measured by Nanoparticle Tracking Analysis (NTA) from the NanoSight product range and Dynamic (DLS) and Electrophoretic (ELS) Light Scattering from the Zetasizer product range within Malvern Instruments. Both NanoSight and Zetasizer are similar because they both rely on the Brownian motion of and light scattering from the particle. Both use the Stokes-Einstein equation and relate diffusion to size (hydrodynamic diameter). In practice, they are quite different, since NTA produces a number-based size distribution and DLS produces an intensity-based distribution. NTA provides particle-by-particle measurement while DLS provides an ensemble measurement. This is further exemplified in the set of experiments shown here. A broad range of characterization information and combination of both NanoSight and Zetasizer systems helped further optimize fabrication and understand the function of liposomes. NTA through NanoSight provided number-based high resolution sizing, accurate distribution profiles, concentration (particles/mL), and fluorescence measurements. DLS provided excellent reproducibility, mean size and PDI measurements over a broad range and non-invasive trend analysis. ELS provided zeta potential as a functionality and stability metric of particles.

Keywords: Instrumentation, Light Scattering, Materials Characterization, Particle Size and Distribution
Application Code: Drug Discovery
Methodology Code: Physical Measurements
Silica as stationary phase is often derivatized with a functional ligand and has a limitation when it comes to breakage of the siloxane bonds at low pH and dissolution of the silica backbone alkaline conditions. Stationary phases that use organosilane reinforced silica has been very popular in liquid chromatography in the last decade as chemically stable materials, allowing medicinal and drug discovery laboratories to analyze using a wide pH range. When organosilane is incorporated in the silica, it changes the physical, and chemical properties due to small hydrophobic patches that appears on the surface together with silanol groups. This combination creates a unique mix-mode material so a stationary phase can be used as a multi-chromatographic mode material, with both hydrophobic and hydrophilic interactions to give orthogonal selectivity. The 100 Å materials were packed and tested in various modes including hydrophilic interaction chromatography (HILIC), normal phase chromatography, supercritical fluid chromatography (SFC) and reversed phase chromatography. The same incorporation of organosilane was also made on 300 Å silica materials for separation of proteins using hydrophobic interaction chromatography (HIC).

**Keywords:** Chromatography, HPLC, HPLC Columns, Supercritical Fluid Chromatography

**Application Code:** Drug Discovery

**Methodology Code:** Liquid Chromatography
Food safety monitoring has become necessary as foodborne diseases are increasing. Work to develop smart sensors and labels to indicate food spoilage or presence of harmful toxins is growing. This presentation will discuss design, development and application of a portable biosensor platform that integrates functional nanoparticles and biomolecules on paper for monitoring food quality and safety. To fabricate the biosensors, we use nanoparticles that have tunable redox activity, optical and catalytic properties and can transduce and catalytically amplify signals in chemical and biological detection schemes involving biomolecules. The presentation will discuss the assembly of nanoparticles and target-specific biomolecules in portable sensing platforms and provide examples of applications for food quality monitoring.

Keywords: Food Contaminants, Food Safety, Nanotechnology, Sensors
Application Code: Food Safety
Methodology Code: Sensors
Portable XRF (pXRF) analyzers have been used for safety since their inception to screen consumer materials for dangerous substances such as Pb in paint, RCRA metals in soil and restricted substances (RoHS) in everyday products. Safety screening helps reduce undocumented hazardous waste and protect consumers, especially children younger than twelve, from being exposed to dangerous materials.

pXRF technology is now being used for safety on food product manufacturing floors. Contaminants are the last thing any manufacturer wants in their final products, but it happens. When they are discovered, pXRF is used to help identify the contaminants and to source their origin for corrective action. This project will demonstrate non-destructive pXRF techniques developed which help food manufacturers quickly and accurately identify metal contaminants and their production floor source.

A lightweight portable XRF spectrometer with 4W Rh X-ray tube, SDD detector and SharpBeam™ geometry for high performance, speed and sensitivity was used to measure elements from Mg (12) to U (92). With touchscreen operation, immediate and easy-to-read results, an internal camera and wireless communication, it was run by battery; although, AC power was possible. Ready-to-go metal/alloy calibrations for near-instant metal and alloy matching were pre-installed at the factory. Spectral fingerprint matching with ARTAX™ PC comprehensive elemental qualitative software was also used.

Data presented will include near-instant pXRF metal or alloy grade matching as well as ARTAX™ spectral fingerprint matching. Methods developed for sample preparation and presentation will be described along with best practices for cataloging production floor contaminant sources, identifying found contaminants and determining their source.

Keywords: Elemental Analysis, Food Contaminants, Portable Instruments, X-ray Fluorescence
Application Code: Food Safety
Methodology Code: Atomic Spectroscopy/Elemental Analysis
The increasing consumption of dietary supplements is well documented. According to Filipiak-Szok and co-workers (Journal of Trace Elements in Medicine and Biology 2015, 30, 54-58) consumption of herbal dietary supplements is also increasing in Europe. Although the European Union (EU) Directive 2004/24/EC of 2011 attempted to put a strict regulation on Herbal Medicinal products about 90% of THM manufacturers in Spain shifted their product to the less regulated food sector (Gaceta Sanitaria 2015, 29, 221-223.). However, contamination of herbal products is a well documented phenomenon in literature. In this work, 16 dietary supplements (mostly herbal supplements) purchased from retail markets in Spain were analyzed for 20 metals (Li, B, Mg, Al, Cr, Fe, Mn, Co, Ni, Cu, Zn, As, Se, Mo, Ag, Cd, Sb, Ba, Tl, and Pb ) by inductively coupled plasma mass spectrometry (ICPMS). The goal of the study was to compare qualities and safety of consumption of randomly acquired herbal supplements from Spain and USA based on the EU and USA reference values. Also, we are interested in identifying factors contributing to differences and similarities of supplements from the two countries. Results of the metal content, estimated daily intake of the metals, the quality and safety of the herbs and chemometric analysis will be presented.

Keywords: Contamination, Elemental Analysis, Environmental Analysis, Food Contaminants
Application Code: Food Safety
Methodology Code: Atomic Spectroscopy/Elemental Analysis
Abstract Text

Popularity and consumption of medicinal herbs is increasing globally. China is perhaps the leading global supplier of herbs utilized as supplement (medicinal and nutritional). The quality and safety of these herbs are affected by their chemical and microbial content. Most quality and safety studies typically analyze commercial (processed) products. Fewer studies focus on crude (unprocessed) samples. Long and coworkers (Journal of Ethnopharmacology 2016, 186, 343-350.) concluded that there is still a knowledge gap in the area of establishing adulterations from traditional drugs. In another review, Saha and coworkers (Phytochemistry Letters 2015, 14, 67 -78) reported heavy metals contamination of medicinal plants from 10 countries (China, Brazil, Egypt, India, Pakistan, Ghana, USA, South Africa, Botswana, and Sudan). In this work, 20 Chinese and Korean herbs (11 crude and 9 processed) purchased from ethnic stores in the USA were analyzed for their metal content by ICPMS. Twenty metals (Li, B, Mg, Al, Cr, Fe, Mn, Co, Ni, Cu, Zn, As, Se, Mo, Ag, Cd, Sb, Ba, Tl, and Pb) were determined in each sample. The goal of the study is to compare qualities, safety, and adequacy of the processed versus crude (unprocessed) herb products for human consumption. Chemometric methods will be applied to the dataset to identify and understand the distinguishing factors between the groups of herbs. Results of the metal content, estimated daily intake of minerals, quality and safety of the herbs and chemometric elucidation of similarities/dissimilarities among the groups will be presented.

Keywords: Contamination, Elemental Analysis, Environmental Analysis, Food Contaminants

Application Code: Food Safety

Methodology Code: Atomic Spectroscopy/Elemental Analysis
Honey is one of the leading exports in Turkey behind olive oil. As a high commodity product, honey has been prone to adulteration. Adulteration of honey is completed by substitution of high fructose corn syrup (HFCS), maltose syrup (MS), beet sugar and other cheap invert sugars. This is a critical issue in the honey industry. Our objective was to develop a rapid untargeted approach to authenticate high-value honey by combining Raman spectroscopy and pattern recognition analysis. Samples (n=85) were collected from Turkish markets. The composition of samples were characterized by reference methods to identify potential adulteration by using sugar analysis by high performance liquid chromatography (AOAC Official Method 977.20). The reason may be that the major content in authentic honey is monosaccharide, e.g., glucose and fructose. Adulterated samples contained various disaccharides like maltose and sucrose. Spectra was collected by using a portable Raman system, analyzed by pattern recognition techniques that included Soft Independent Model of Class Analogies (SIMCA) to develop classification algorithms. Authentic honeys formed distinct clusters allowing the evaluation of commercial samples from local markets in Turkey showing some prevalence of adulteration. Spectral differences responsible for the separation of classes were associated with the signals of authentic and adulterated honey showed characteristics bands around 351, 425, 517, 592, 629, 705, 778, 824, 865, 915, 981, 1065, 1127, 1264, 1373 and 1461 cm⁻¹. New generation portable Raman devices provided a viable tool for chemical profiling of Turkish honey samples allowing for the rapid, “in-field”, and reliable authentication of food ingredients, making it a great alternative to time-consuming traditional testing methods.

Keywords: Food Identification, Food Safety, Quality, Vibrational Spectroscopy
Application Code: Food Safety
Methodology Code: Vibrational Spectroscopy
**Abstract Text**

SERS is a powerful technique for food inspection because of its readiness, sensitivity, and minimum sample preparation requirements. [1] Milk is a vulnerable target for contamination. In this work, we demonstrate a reliable SERS method for detecting toxins in milk focusing on brodifacoum, an anticoagulant rodenticide and sodium fluoroacetate, also commonly known as 1080. Surface-enhanced Raman spectroscopy is an advanced Raman technique for ultrasensitive detection of chemical and biological species. Liquid milk presents further challenges due to the complex colloidal nature of milk itself; producing much weaker Raman scattering SERS.

Therefore, we worked on an omniphobic surfaces platform which has the potential to deliver near 100% analyte concentration by constant contact angle drying (and therefore no contact line pinning). Such omniphobic SERS substrate, a so-called Slippery liquid-infused porous surfaces (SLIPS) was recently reported. [2] SLIPSERS method coupled with the dilution of rodenticide spiked milk samples and then extraction with a mixed solvent of methanol: water (3:1) was performed for rodenticide detection. [3] All the spectra were taken on an in-house Raman set up based on a FERGIE spectrometer using 532 nm excitation wavelength (with 2-3 mW laser power) focused onto the sample using a 40 ×, 0.65 NA objective. A series of diluted concentrations of each rodenticide ranging from 8-fold dilution to 1600-fold dilution was conducted. And there is a good linear relationship (R2=0.9897) in this concentration range. The approach adopted in this work can be effectively applied for the detection of various molecule in complex chemical and biological matrices. A critical challenge is to achieve high specificity, high throughput, and trace-level detection. [4]

**Keywords:** Food Contaminants, High Throughput Chemical Analysis, Surface Enhanced Raman Spectroscopy, Tox

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

**Methodology Code:** Vibrational Spectroscopy
Determination of B-vitamins in Infant Formulas by Capillary Electrophoresis-Tandem Mass Spectrometry: Focus on Sample Preparation

B-vitamins belong to water soluble vitamins that humans cannot synthetize (except for pyridoxine) and have to be provided from the food. For many infants, patients after surgery, and elderly people, infant formula and related nutritionals are the only source of B-vitamins and other important nutrients, and thus, it is necessary to ensure their content. The determination of B-vitamins is still mostly based on individual determination by microbiological assays, which can be costly and time consuming. A CE-MS/MS is a highly efficient separation method whose potential was already shown in simultaneous determination of B-vitamins in pharmaceutical samples [1]. However, infant formula and related nutrients represent complex sample matrix, which require different sample treatment before analysis. The aim of this work was to develop a simple and fast procedure for the determination of B-vitamins in these products by CE-MS/MS. Three sample treatments were compared. The sample treatment A was based on previously described procedure [2] and modified in order to apply the methodology for CE-MS/MS. The treatment B was based on the original QuEChERS method, and the treatment C was based on extraction of B-vitamins with formic acid and acetonitrile. The best recoveries were obtained with the sample treatment C, yielding from 91 and 102% for folic acid (B9) and pantothenic acid (B5), respectively. Extracted B-vitamins were separated in a PVA-coated capillary using 0.05 M formic acid as background electrolyte and detected by ESI-MS/MS. The CE-MS/MS method showed low LODs and LOQs, in the ranges of 1–2 ng/mL and 3–8 ng/mL, respectively. The range of the correlation coefficients of the calibration curves was 0.996–0.999. The method was successfully used to determine B vitamins in commercial samples of infant formula.

References:
Here I present a mobile instrumentation platform based on a smartphone using its built-in functions for colorimetric diagnosis where a smartphone app digitizes the colors of the colorimetric sensor array. First, the concept is proved by pH test paper which measures proton concentrations with a smartphone. A more sophisticated application was made with a multi-analyte sensor array. A conventional colorimetric sensor array consists of multiple paper-based sensors, and reports the detection results in terms of color change. The color changes are normally recognized by naked eyes, which may cause uncertainties by the personal subjectivity and the surrounding conditions. Solutions to it have been severally sought in smartphone technology replacing a spectrometer. Advancing the technique, our poster specifically focuses on development of a practical app for the immediate point-of-care (POC) multi-analyte sensing without additional devices. First, the individual positions of the sensors are automatically identified by the smartphone. Second, the colors measured at each sensor are digitized based on the correction algorithm. Third, the corrected colors are converted to concentration values by the pre-loaded calibration curves. All through these sequential processes, the sensor array taken in a smartphone snapshot undergoes the laboratory-level spectrometry. The advantages of the inexpensive and convenient paper-based colorimetry and the ubiquitous smartphone are tied to achieve the ready-to-go POC diagnosis.

Keywords: Portable Instruments, Sensors, Software
Application Code: Biomedical
Methodology Code: New Method
Two simple, sensitive and specific HPTLC-DAD methods were developed for the determination of febuxostat (FEB) individually, and simultaneously with diclofenac potassium (DIC) in human plasma. The first method presents the first HPTLC attempt for FEB determination in human plasma. FEB was separated (at RF=0.67) on precoated silica gel 60 GF254 plates with ethyl acetate- methanol-water (9: 2: 1, v/v) as mobile phase. It was quantified at its \( \lambda_{\text{max}} \) (315 nm), and the calibration plot was linear between 0.5-7 \( \mu \)g mL\(^{-1} \). The second method is considered the first attempt for the simultaneous determination of FEB and DIC in human plasma. It used petroleum ether-chloroform-ethyl acetate-formic acid (7.5: 1: 2.5: 0.25, v/v) as mobile phase. Both drugs were separated at RF of 0.35 and 0.58 for FEB and DIC, respectively. Both FEB and DIC were quantified at their isoabsorptive point (289 nm), and the calibration plots were linear between 0.6-7 and 0.4-8 \( \mu \)g mL\(^{-1} \) for FEB and DIC, respectively. Sample preparation was performed by liquid-liquid extraction using diethyl ether. Both methods did not record any interference from plasma matrix or the studied drugs’s metabolites, and were fully validated according to the FDA guidance. The proposed methods provide very simple, rapid and cheap alternatives that might be attractive for the future pharmacokinetic and bioavailability studies of FEB and/or DIC.

**Abstract Text**

Two simple, sensitive and specific HPTLC-DAD methods were developed for the determination of febuxostat (FEB) individually, and simultaneously with diclofenac potassium (DIC) in human plasma. The first method presents the first HPTLC attempt for FEB determination in human plasma. FEB was separated (at RF=0.67) on precoated silica gel 60 GF254 plates with ethyl acetate- methanol-water (9: 2: 1, v/v) as mobile phase. It was quantified at its \( \lambda_{\text{max}} \) (315 nm), and the calibration plot was linear between 0.5-7 \( \mu \)g mL\(^{-1} \). The second method is considered the first attempt for the simultaneous determination of FEB and DIC in human plasma. It used petroleum ether-chloroform-ethyl acetate-formic acid (7.5: 1: 2.5: 0.25, v/v) as mobile phase. Both drugs were separated at RF of 0.35 and 0.58 for FEB and DIC, respectively. Both FEB and DIC were quantified at their isoabsorptive point (289 nm), and the calibration plots were linear between 0.6-7 and 0.4-8 \( \mu \)g mL\(^{-1} \) for FEB and DIC, respectively. Sample preparation was performed by liquid-liquid extraction using diethyl ether. Both methods did not record any interference from plasma matrix or the studied drugs’s metabolites, and were fully validated according to the FDA guidance. The proposed methods provide very simple, rapid and cheap alternatives that might be attractive for the future pharmacokinetic and bioavailability studies of FEB and/or DIC.

**Keywords:** Analysis, Biological Samples, Chromatography, Thin Layer Chromatography
New Method

Tri-Nucleotide Rolling Circle Amplification - A Novel Method for the Detection of RNA and DNA

Most natural DNA and RNA are devoid of long Tri-Nucleotide (TN) stretches of sequences that lack one specific nucleotide (Missing Nucleotide (MN)). Here we developed a novel method that is based on Rolling Circle Amplification (RCA), in which the TN-information of short TN stretches in DNA or RNA is sequence-specifically recognized, transferred, extended, amplified and detected by Padlock probes that consist entirely of nucleotides complementary to the nucleotides present in the target sequence (complementary TN-information). Upon specific head-to-tail annealing and ligation to the TN-target sequence, these Padlock probes represent extended complementary TN versions of the target sequence that can be further amplified by Tri-Nucleotide Rolling Circle Amplification (TN-RCA) into linear concatemeric long ssDNA. Since during TN-RCA the MN (as dNTP) is not added to the reaction mixture during polymerization, only the correctly ligated circular Padlock will amplify, and background amplification will not occur with endogenous RNA or DNA (which mostly would require the presence of all four dNTP). Therefore, in the absence of non-specific amplification, various labeled dNTP can be added to the TN-RCA reaction that enables the separation, isolation and detection of the amplified concatemeric linear ssDNA. Here we evaluate the TN-RCA method with RNA/DNA derived from Zika, Noro and human papilloma virus (HPV), using visual detection methods suitable for resource-limited point of care settings including lateral flow assay on paper dipsticks, microtiter plates, and syringe/filter devices. TN-RCA is a novel isothermal amplification technique that can be used for rapid and sensitive sequence-specific detection and diagnosis of natural and synthetic DNA or RNA containing TN stretches (genomic DNA, bacteria, virus, mutations, polymorphisms, DNA methylation) with low background in short time.

Keywords: Bioanalytical, Biomedical, Biosensors, Fluorescence
Application Code: Biomedical
Methodology Code: New Method
Recent Research Developments in the Certification of Organic Reference Materials by 1H, 31P and 19F Quantitative NMR at the Highest Metrological Level

Abstract Text
Quantitative NMR (qNMR) spectroscopy has become an important tool for the content determination of organic substances and the quantitative evaluation of impurities. Since the signal intensity is directly proportional to the number of protons contributing to the resonance, qNMR is considered as a relative primary method. Sigma-Aldrich R&D demonstrated the validity, robustness and precision of the 1H qNMR technique through the optimization of High-Performance qNMR (HP-qNMR®) to its maximum level of accuracy using metrological weighing equipment and a specially designed experimental setup for the certification of organic compounds with combined, expanded uncertainties down to 0.1%. The implementation of qNMR in new application fields (e.g. metabolomics, biomarker discovery, physiological pathways) brings along more complex molecules and systems, thus making the usage of 1H qNMR challenging. The use of other NMR active nuclei provides an elegant solution for which new qNMR standards are required. Therefore, we developed two additional classes of qNMR Certified Reference Materials (CRM), based on different NMR active nuclei. Figure 1 shows the certification concepts for 31P and 19F qNMR CRM to achieve traceability to the SI by using primary Reference Materials from the National Institute of Standards and Technology (NIST) and the National Metrology Institute of Japan (NMIJ).

Keywords: NMR, Quantitative
Application Code: Food Identification
Methodology Code: New Method
Large quantities of radioactive contaminated water have been produced in melted-fuels cooling system in Fukushima Daiichi Nuclear Power Plant. The demand of rapid radionuclide analysis has been increasing. Traditional radiometric analysis for beta-ray emitting nuclides, in particular $^{90}$Sr is complicated and consumes two weeks and more. Similarly, $^{129}$I is also a beta-ray emitting nuclide and is employed as tracer of environmental dynamics. Recently, a cascade type ICP-MS was developed for the analysis of $^{90}$Sr within 20 minutes; however multi-nuclides analysis was difficult in that method. In this study, new system was constructed using this cascade type ICP-MS and a split line system. In the proposed method, the sample solution splits two directions in the flow path. One was introduced into a solid phase extraction column for Sr analysis (SPE line). The other flow was directly introduced to ICP-MS for I analysis (split line). Both flows were sequentially introduced to ICP-MS and measured. These values of the limit of detection (LOD) can be lower using ultrasonic nebulizer (USN) for Sr and coaxial nebulizer for I, respectively. From this reason, we have fabricated new hybrid chamber for two-nuclides injection. In the simultaneous quantification, the chromatographic peak area of $^{90}$Sr and the average signal intensity of $^{129}$I were employed. The LODs were 0.97 and 2.0x10^{-3} Bq/L for $^{90}$Sr and $^{129}$I, respectively (sample volume: 10mL), with 13.2% and 10.1% of repeatability (RSD (n=3)). In the application to diluted radioactive contaminated water could be obtained the quantification value, respectively.

Keywords: Environmental Analysis, Flow Injection Analysis, ICP-MS, Nuclear Analytical Applications

Application Code: Nuclear

Methodology Code: New Method
Transporters in the intestinal epithelium play a critical role in the absorption of drugs from the intestine for delivery into the bloodstream. At present, a human colon carcinoma cell line, Caco-2, is used to model drug transport and toxicity. Caco-2 cells are largely undifferentiated cancer cells and exhibit genomic instability—properties that may not always reflect in vivo physiology for examination of drug toxicity and transport. A cell monolayer derived from primary colon tissue was cultured on a biomimetic scaffold to model drug transport and toxicity. Isolated mouse intestinal epithelial cells cultured on the scaffold proliferated to yield a contiguous monolayer predominantly comprised of absorptive colonocytes within 5-6 days. The measured trans epithelial electrical resistance (>300 cm²) and Lucifer yellow permeability (<1x10⁻⁷ cm s⁻¹) of the monolayers suggested the presence of a tight monolayer that would be of value in drug transport studies. The mRNA expression pattern for transporters and pumps in this monolayer culture was similar to that measured from freshly isolated tissue. Monolayer transport function was evaluated using both rhodamine 123 (Rh123, a substrate for P-glycoprotein) and riboflavin (transported by solute carrier family transporters). Rh123 was transported towards the luminal side (Papp, efflux/Papp, influx =7) of the monolayer and was blocked by verapamil, a known inhibitor of P-glycoprotein. Riboflavin was also transported across the monolayer and saturation of the transporter was observed at high riboflavin concentrations. These primary intestinal epithelial monolayers will find wide utility in drug transport studies.
New Method

New Method for Quantifying Cell Viability in 3D Tumor Models with Quantitative PCR

Cell viability assays are commonly used in cancer research and drug screening to evaluate cytotoxicity. Viability is indirectly determined by absolute quantification of cellular responses such as mitochondrial activity, ATP concentration, or fluorescent protein expression using a standard curve prepared under standard laboratory conditions. However, this curve only accounts for a single culture condition and cannot account for the heterogeneous cellular environment within a 3D tumor model. The tumor microenvironment is complex and composed of multiple gradients including: oxygen, nutrients, and pH. Commonly used viability assays do not produce accurate readings across these gradients—specifically oxygen and glucose—which impact the cellular responses used to quantify viability. Cells near the tumor core will experience hypoxia and limited glucose levels, which will result in altered metabolic activity, ATP production, and protein expression when compared to cells located in the outer normoxic regions. We propose a new cell viability assay that quantifies the number of cells present in a sample by quantitative polymerase chain reaction (qPCR). This assay will produce accurate measurements across oxygen and glucose gradients because genomic DNA copy number in viable cells is not affected by changes in cellular environment. We utilize a photoactivatable DNA binding molecule that selectively binds to the DNA of a dead cell and inhibits its qPCR amplification. When combined with a non-treated sample, this assay enables a quantitative measurement of both live and dead cells, providing information not generated by current viability assays. We have optimized our assay with respect to DNA measurement, biomolecule cell treatment and reaction conditions, sample extraction technique, and qPCR primer and target validation. This assay will enable cell culture researchers to more accurately study cellular phenotypes in 3D tumor models.

Keywords: Biomedical, Method Development, Quantitative, Toxicology
Application Code: Bioanalytical
Methodology Code: New Method
Quantitative NMR (qNMR) spectroscopy has become an important tool for the content determination of organic substances and the quantitative evaluation of impurities. Some years ago, we demonstrated validity, robustness and precision of the 1H qNMR technique through the optimization of High-Performance qNMR (HP-qNMR®) to its maximum level of metrological accuracy. The implementation of qNMR in new application fields (e.g. metabolomics, biomarker discovery, physiological pathways) brought along more complex molecules and systems that required additional classes of qNMR Certified Reference Materials (CRM), based on different NMR active nuclei, namely phosphorous and fluorine. With the rise of qNMR in pharmaceutical industry and testing laboratories, there is a strong need for proficiency testing and inter-laboratory comparison studies in that field. Unfortunately, only a very limited number of providers is present, which all do not possess the ISO 17043 certification. Therefore, we did set up a completely new qNMR proficiency testing scheme using our in-house developed qNMR standards, which combines 1H, 31P and 19F qNMR measurements and allows a performance assessment with regard to each individual nucleus (see Figure 1). The participants were selectively chosen from metrological institutes, pharmaceutical companies and testing labs in order to have representative subgroups for a detailed analysis, of which first results will be presented.
New Method

Dynamic Methods to Produce Calibration Gas Mixtures: How to Validate the Dilution Systems?

Gas Mixtures are required in numerous applications as Environment, Engine Testing, Research, or Healthcare. Calibration Gas Mixtures are necessary to control or to calibrate analyzers. It is not always possible to get Calibration Gas Cylinders for this purpose and producing on-site (real time) the Gas Mixtures at the right concentration is more comfortable for the end user. Without taking in account the constraints of gas pressure and flow or gas purity, the problem is how to validate these Gas Mixtures produced by dilution system, especially if they are used as Calibration Gases or required in critical process.

Validation and Verification of Gas Mixtures produced by dilution systems are under discussion in the working group 5 of ISO 158 for the revision of the ISO 6145-1. This standard promotes two ways to assess the calibration of dilution systems: one by flow calibration of each element of the dilution system and the other considering the mixing system as global entity where the generated gas mixture is validated by comparison with reference gas mixtures. The second approach corresponds to the global method which is investigated in this presentation. The purpose is to demonstrate that few reference gas mixtures are sufficient to validate a dilution system covering a large range of gas concentrations produced by the mixing system. The method developed for this purpose offers different simple possibilities to validate and to control the dilution system. Some examples are included in the presentation making easy to understand how to apply the global approach and what are the advantages and limits of this method.

Keywords: Environmental, Sampling, Specialty Gas Analysis, Validation
Application Code: Validation
Methodology Code: New Method
Fluorescence biomarkers have widespread application in high-throughput and high-content screening technologies which are widely used in cancer research for identifying potential drug candidates and disease detection. However, most biomarkers are specifically designed to recognize a single target, so the selection of biomarkers requires prior-knowledge about the target and identification results are affected by prior knowledge. As a trade-off between accuracy and computational complexity, for most current biomarkers, only one variable such as the intensity of a single wavelength or the average of certain wavelengths from the emission spectrum is collected and analyzed, and the information contained in the emission shape cannot be utilized.

Herein we report a new conjugated polymeric biomarker P-C-3 and a methodology for analyzing its fluorescence spectral shape which is strongly depended on the local/ionic environment. This fluorescence biomarker is able to classify 12 kinds of nucleoside phosphates (NTP, NDP, and NMP; N = A, C, G, and U) in 1 minute and achieves 100% classification accuracy. By analyzing the fluorescence spectral shapes, we demonstrated that fluorescence spectral shapes of P-C-3 are sensitive to both the charge and the structure of analytes. We also developed an algorithm to select useful features from the fluorescence spectrum to reduce computational complexity and prevent overfitting. By just measuring the normalized intensity of these 3 selected wavelengths by the plate reader, we are able to achieve 100% classification accuracy for the 12 kinds of nucleoside phosphates by linear discriminant analysis (LDA). This is the first classification method to utilize the information contained in fluorescence spectrum shapes of a polymeric biomarker. With this methodology, developing a universal biomarker for high-throughput classification and clustering of cell type and phenotype becomes possible, which is also under investigation.

Keywords: Biosensors, Fluorescence, High Throughput Chemical Analysis, Statistical Data Analysis
Application Code: Bioanalytical
Methodology Code: New Method
The recent surge in plasma-source development within the analytical sciences has come from the realization that atmospheric-pressure discharges can be useful for excitation and/or ionization of a broad range of species. This impact has been most notable in mass spectrometry where plasmas have been used for the formation and direct detection of bare atomic ions, small organic molecules, and even intact labile biopolymers. As such, these plasmas can be viewed as multimodal sources whereby many types of analytical information can be obtained from a plasma source within a single experiment. Recently, our group has been exploring the range of analyses capable by these atmospheric-pressure discharges, such as the solution-cathode glow discharge (SCGD) and flowing atmospheric-pressure afterglow (FAPA). In addition, we have been exploring plasma-based methods to generate comprehensive chemical maps (or “images”) of solid samples with high spatial resolution.

Here, we will present our recent work towards the development of a multimodal chemical imaging apparatus capable of providing simultaneous molecular and elemental information from the exact same spatial location (i.e. each pixel). The high spatial resolution is achieved through focused laser sampling (i.e. ablation) of a solid sample. Meanwhile, the aforementioned plasma sources are used to excite and/or ionize atoms or molecules in the ablated aerosol. Optical emission and mass spectrometry are then used to obtain elemental and molecular information, respectively, from the laser-sampled location. In addition, light from the laser-induced plasma during the ablation event can be measured (i.e. laser induced breakdown spectroscopy) to provide additional information on the elements present in the sampled spot. The design of these instrumental configurations will be discussed in detail along with preliminary analytical figures-of-merit.

**Keywords:** Atomic Emission Spectroscopy, Imaging, Isotope Ratio MS, Mass Spectrometry

**Application Code:** General Interest

**Methodology Code:** New Method
Man-made nanoparticles (NPs) have found wide application across a variety of fields. However, people are becoming increasingly concerned about NPs potential human health risk and environmental impact. Although both acute and chronic toxic effects of NPs have been broadly reported using in vitro and in vivo models, controversial conclusions and abrupt rejections on nanotoxicities still exist. In the conventional study, advanced analytical techniques are used to assess the cell viability, intracellular ROS accumulation, mitochondrial inner membrane potential, as well as the Apo-BrdU TUNEL apoptosis assay and compared with the control cells. In this study, a novel technique has been developed to assess the cell viability when the cells were exposed to different types of nanoparticles. An accurately controlled width of gap was created in the middle of the seeded cells on a poly-lysine coated cover slip and an accurately measured amount of nanoparticles were dosed in the middle of the gap as a fine strip. The cell growth rates were evaluated by measuring the gap width changes at different amounts of nanoparticles and at different exposure times. In this experiment, a series of different nanoparticles were studied, such as silicon dioxide (SiO2), titanium dioxide (TiO2), Cerium (IV) oxide (CeO2), and borate-based glass fibers. The results showed that some nanomaterials enhanced cell growths while some of them decreased the cell viability significantly. The pHs at different distances from the nanoparticles in the gap were measured by using our newly developed micro-pH probe. The detailed experimental procedure and results will be presented at the conference. In conclusion, this study provides a simple and fast assessment of cytotoxicity of nanomaterials before quantitative data are collected for intracellular ROS accumulation, mitochondrial inner membrane potential and others by using advanced analytical techniques.
The desoxy phenethylamine analogues in this study represent a combination of alkyl side-chain and cyclic amines (azetidine, pyrrolidine, piperidine and azepane) to yield a set of molecules of identical elemental composition as well as major mass spectral fragment ions (base peaks) of identical elemental composition. These desoxy phenethylamine analogues of the aminoketone designer drug, 3,4-methylenedioxy-pyrrovalerone (MDPV) related to the natural product cathinone were prepared from piperonal (3,4-methylenedioxybenzaldehyde) via the intermediate precursor ketones. The aminoketones and the desoxy phenethylamine regioisomers were each separated in capillary gas chromatography experiments using an Rxi®-17Sil MS stationary phase with the aminoketones showing greater retention than the corresponding desoxyamines.

The electron ionization mass spectra for the aminoketones as well as the desoxy phenethylamines yield equivalent m/z 126 regioisomeric iminium cation base peaks. However, the product ion spectra allow for the differentiation of the m/z 126 iminium cations containing the various ring size cyclic tertiary amines and alkyl side-chain. The product ions from the m/z 126 iminium cation produced by the desoxy phenethylamines containing the azetidine, pyrrolidine, piperidine and azepane cyclic amines occur at m/z 70, 84, 98 and 72, respectively.

The vapor phase infrared spectra for these desoxy phenethylamines show doublet absorption bands at 1489 cm⁻¹ and 1442 cm⁻¹ characteristic for the 3,4-methylenedioxy aromatic ring substitution pattern and the unsymmetrical nature of these doublet absorption bands indicates the lack of a carbonyl group at the benzylic position of the alkyl side-chain.

Keywords: Capillary GC, Forensic Chemistry, GC-MS, Ion Trap
Application Code: Drug Discovery
Methodology Code: Gas Chromatography/Mass Spectrometry
Crime is known to concentrate into "hotspots": small areas with locally high crime rates. Crime also exhibits "near-repeat" behavior, where recent crimes tend to locally increase the rate of crime for few days or weeks, perhaps due to retaliation or repeat offenses. Tools to understand the causes and dynamics of these hotspots are limited and don't include near-repeat behavior, while analysis of near-repeats typically ignores hotspots. We propose a spatio-temporal statistical model which accounts for both spatial and temporal variation in crime by modeling hotspots, near repeats, and leading indicator crimes, and demonstrate its performance on a large dataset of crimes in Pittsburgh, Pennsylvania, showing its use for testing criminological theories and predicting crime.
The application of Next Generation Sequencing (NGS) for the analysis of mitochondrial (mt) DNA, STRs, and SNPs has demonstrated great promise for challenging forensic specimens, such as degraded, limited, and mixed samples. Target enrichment using probe capture rather than PCR amplification offers advantages for analysis of degraded DNA since two intact PCR primer sites in the template DNA molecule are not required. Furthermore, the same shotgun library prepared from a limited DNA source can be enriched for mtDNA sequences as well as nuclear markers by sequential hybrid capture with the relevant probe panels. We have developed probe capture assays targeting the entire mtgenome and 426 nuclear SNPs for massively parallel sequencing of highly degraded and mixed DNA samples. Here, we demonstrated the use of this strategy in the analysis of limited, degraded, and mixed samples. The nuclear SNP probe capture NGS assay was tested on size selected samples over a range of DNA input amounts and >99% of SNPs were recovered and sequenced including from samples 75bp and as low as 0.5 ng. With the mtgenome assay, ~100% coverage was obtained from highly compromised DNA samples, including highly degraded DNA from bones and touch DNA recovered from spent cartridge casings. We also applied the probe capture NGS systems to the analysis of mixed DNA samples with as low as 10% minor contributor. To improve mtDNA mixture analysis, we utilized phylogenetic and read frequency based data analysis approaches to resolve the major and minor contributors. Finally, the results obtained on individual telogen hairs demonstrated the potential of probe capture NGS analysis for both mtDNA and nuclear SNPs for challenging forensic specimens.

**Keywords:** Biomedical, Forensics, Genomics, High Throughput Chemical Analysis

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

**Methodology Code:** New Method
Analysis of trace amounts of drugs is important in a variety of situations, including forensic casework. Here, a method for the facile, rapid collection of traces of drugs from a variety of porous and non-porous surfaces, including fabrics, is detailed. A small amount of extraction solvent, including an internal standard, is applied to the fabric surface, followed by application of a patterned absorbent disk which resorbs much of the extraction solvent along with dissolved traces of any drug present. Over half of the extraction solvent is recovered in fifteen seconds from many natural and synthetic fabrics, with weights ranging from 64 to 374 mg inch\(^{-2}\), by pressing a half-inch diameter patterned glass fiber membrane disk to the wetted area. The patterned disk is then placed in a standard OpenSpot holder of a Direct Analysis in Real Time (DART) mass spectrometer with a data collection time of one to two minutes. Semi-quantitation of low microgram levels of drugs is achieved by comparison of spectra to those from a standard control disk. DART signal generation from the absorbent disks is much longer lived (>2 minutes) than from commercially available OpenSpot cards (~10 seconds) due to the much larger sample capacity of the glass fiber membrane in comparison to the wire mesh of the OpenSpot cards. In an intermediate precision study with four analysts over four days, the average recovery from 190 µg methamphetamine spiked fabric was 8.6 ± 3.2 % indicating that sub-milligram traces of drugs are reliably extracted and measured from fabric. Complete recovery of traces of drug from fabric is not expected since some of the internal standard solution remains in the fabric and the disk may not be placed exactly over the area that was spiked with the drug.

**Keywords:** Drugs, Forensics, Forensic Chemistry, Trace Analysis

**Application Code:** Homeland Security/Forensics

**Methodology Code:** Mass Spectrometry
Combining Cranial and Dental Data to Improve the Estimation of Ancestry in the Forensic Context

Ancestry estimation is central to the construction of a biological profile in forensic anthropology; however, the Daubert standard of evidence requires that methods used in the forensic sciences be testable and have known rates of error. The methods employed in the analysis of cranial morphoscopic and dental morphological traits largely do not meet that standard. Furthermore, no method exists for producing an ancestry estimate from the combination of different datasets.

Cranial and dental traits were examined in a sample of 693 individuals from various ancestry groups representative of U.S. populations. Variables were removed from further analyses if they did not differ significantly among ancestry groups or were highly correlated with other variables. The remaining variables were used to produce classificatory models applicable to the estimation of ancestry.

Ancestry was estimated correctly in 67%-84% of cases. In general, models that combined cranial and dental data outperformed models based on a single data source; although, the accuracy gained by combining methods was not statistically significant across all groups. The combined data models showed the most marked improvement in estimating the ancestry of Hispanic individuals, suggesting that the cranium and dentition provide different information with regard to ancestry.

The methods used to produce ancestry estimates in this research comply with the Daubert standard of evidence, making them applicable to modern forensic casework. Additionally, the results highlight the improvement to ancestry estimation by combining data from different regions of the skeleton, and the utility of the dentition in forensic estimates of ancestry.

Keywords: Forensics, Identification, Method Development, Statistical Data Analysis
Application Code: Homeland Security/Forensics
Methodology Code: New Method
High-Resolution Melt can be used to Quickly Identify New Loci for Body Fluid Identification Using DNA Methylation Melt Analysis

This project uses High Resolution Melt analysis to quickly screen new potential loci for DNA methylation analysis, with the possibility of creating a confirmatory tool for body fluid identification. DNA methylation is a natural process involving the addition of a methyl group to the 5’carbon of cytosines in a dinucleotide cytosine-guanine (CpG) pair. Whole genome analysis of CpGs using a commercially available array can provide additional loci of interest but it must be followed by a confirmatory PCR-based assay. HRM constitutes a great experimental tool to perform screening for multiple genome locations quickly and has a great potential to be used as a standard method in forensic laboratories to discriminate body fluids.

Blood, buccal swabs, vaginal and semen samples were collected from volunteers. DNA was extracted using the EZ1® DNA Investigator kit (Qiagen, CA) and the BioRobot® EZ1 (Qiagen, CA) and then bisulfite modified using the EpiTect® Fast DNA Bisulfite Kit (Qiagen, CA) in order to convert the unmethylated cytosine to uracil which will cause amplicons with low GC content to melt at a lower temperature than amplicons with high GC content. Primers specific for the CpG of interest were designed using online tools. Bioinformatic analysis was performed using R software to determine relevant CpGs to discriminate blood, saliva and vaginal epithelia. Real-time PCR reactions were performed using either the Epitect® HRM kit (Qiagen, CA) or an optimized master mix on a Rotor Gene 6000 real time instrument (Qiagen, CA). To date this approach allowed us to obtain a 71% success in identifying new CpGs in silico. For example, from 7 CpGs identified as potential blood markers, 5 proved to show a difference in their TM for blood when compared to saliva, semen and vaginal epithelia, using HRM. Due to the high throughput of HRM, we are able to quickly screen loci of interest with an analytical tool appropriate for casework in forensic laboratories.

Keywords: Bioanalytical, Bioinformatics, Biological Samples, Forensics
Application Code: Homeland Security/Forensics
Methodology Code: New Method
Signal amplification via enzyme-assisted target recycling (EATR) offers a powerful means for improving the sensitivity of DNA detection assays, but it has proven challenging to adopt EATR into the aptamer-based assays for small-molecule detection, because insensitive target response of aptamers. Here, we describe a general approach for the development of rapid and sensitive, EATR-amplified small-molecule aptamer sensors using cooperative binding split aptamers (CBSAs). CBSAs contain two target-binding domains, and exhibit enhanced target response compared with single-domain split aptamers. We introduced a duplexed C3 spacer between the two binding domains that offers an ideal substrate for exonuclease III cleavage, enabling EATR signal amplification. As a demonstration, we engineered a CBSA-based EATR fluorescence assay to detect dehydroisoandrosterone-3-sulfate (DIS) in urine samples. This assay achieved a 100-fold enhancement in target sensitivity relative to a non-EATR-based assay, with a detection limit of 1 nM in 50% urine. We further developed an instrument-free colorimetric assay employing EATR-mediated aggregation of CBSA-modified gold nanoparticles for rapid detection of low micromolar concentrations of cocaine. Based on the generalizability of CBSA engineering and the robust performance of EATR in complex samples, we believe that such assays should prove valuable for detecting small-molecule targets in diverse fields.
The incidence rate of violent crimes in the U.S. follows a similar developmental trajectory as neurobehavioral metrics of impulsivity and aggression; rapidly increasing following puberty and peaking at ages 17-19 before leveling off in young adulthood. Environmental stressors including, but not limited to, factors such as family conflict, exposure to violence and deviant social influences have been suggested to alter the development of healthy brain networks, thereby predisposing adolescents towards adverse outcomes. The dual systems model of adolescent brain development specifies individual differences in outcomes may be explained by the developmental timing of increased risk/reward-related neural activity in the striatum relative to the onset of maturation of inhibitory control circuits in the prefrontal cortex. In this work, we provide evidence for these claims using functional Magnetic Resonance Imaging (fMRI) to characterize the maturation of striatal and prefrontal cortical networks. 135 youths (ages 11-14) were assessed for deviant social behavior, environmental stressors, drug use and mental health at three time points spaced 18 months apart from 2011-2017. Striatal-cortical network connectivity increased as a function of age, indicating healthy brain development on average in the sampled group (p<0.001). Individual differences in network connectivity were explained by behavioral phenotypes and social influences. The strength of striatal-prefrontal cortical connectivity was revealed to be weaker in individuals exhibiting future antisocial behavior (r=-0.28; p < 0.003), early emotional disturbances (r=-0.18; p < 0.05) and deviant peer relations (r=-0.19; p < 0.05). Overall, our results demonstrate the potential utility of brain-based biomarkers for predicting, understanding and potentially preventing the emergence of youth antisocial behavior.

Keywords: Biomedical, Data Mining, Neural Network, Pattern Recognition
Application Code: Biomedical
Methodology Code: Magnetic Resonance
Infrared thermal imaging is an evolving nondestructive method useful for identifying local differences in thermal conductivity or regions of plastic strain from subsurface defects in materials. In the case of vehicle and firearm serial numbers, these defects are the underlying deformations that result from the stamping and laser engraving processes. This study utilizes lock-in thermography (LIT) in combination with multivariate data analysis as an alternative to chemical etching; a destructive method currently used to recover defaced serial numbers. The process involves several unique aspects, each of which works to overcome some pertinent challenges associated with the recovery of defaced serial numbers. The thermal differences captured in the infrared images are quite small and not readily visible due to surface irregularities and environmental factors. As such, further enhancement is usually needed to identify and evaluate the subtle variations. Principal component analysis (PCA), a multivariate technique that transforms a dataset of possibly correlated variables into a set of new uncorrelated variables, is employed on the data collected to enhance these variations and aid the recovery of the numbers. Statistical measures are utilized to independently verify and match the recovered numbers to non-defaced equivalents in a pristine library. Prior to computing similarity measures between a library number image and a defaced score image, the images are decomposed to Zernike moment vectors using Zernike polynomials. These vectors contain image features describing shape characteristics of an image. Fusion rules are then applied to the resultant measures to achieve a consensus as to the identification. Results are presented for known defaced numbers on several samples as well as recovery of the defaced serial number on a stolen motorcycle.

Keywords: Chemometrics, Data Analysis, Forensics, Infrared and Raman
Application Code: Homeland Security/Forensics
Methodology Code: Thermal Analysis
Forensic evidentiary backlogs are indicative of the growing need for cost-effective, high-throughput instrumental methods. One such emerging technology that shows high promise in meeting this need, while also allowing on-site investigation, is portable mass spectrometric instrumentation, particularly that which enables the coupling to rapid, ambient ionization methods. In this work, a comprehensive analytical validation of a portable mass spectrometer (MS) featuring a simplified paper spray ionization (PSI) source is undertaken, examining aspects such as spectral accuracy, error rates amongst diverse user classes, reproducibility and method robustness. As such technology is intended for field usage by non-technical operators, an extensive investigation of environmental ruggedness was conducted, observing the effect of ambient temperature, wind speed/direction and relative humidity on collected spectra and associated error rates. While portable MS systems have the potential to serve as a flexible investigative tool during law enforcement activities, the underlying legal implications of evidentiary data require the discretion of practitioners to ensure both lawful and ethical usage. Demonstrated in this work are usage scenarios relevant to policing, premising the legality of more abstract applications (such as latent fingerprint screening on identifying materials to establish probable cause in traffic stops) through a review of current search and seizure law. A proactive examination of economic impact regarding implementation of portable MS systems in law enforcement is also presented, weighing the potential cost-savings and enhanced investigatory capabilities afforded against traditional, laboratory-based evidence processing. This research serves to help inform and guide criminal justice decision-makers in regards to the potential adoption of portable, forensic instrumentation.

**Keywords:** Forensic Chemistry, Mass Spectrometry

**Application Code:** Homeland Security/Forensics

**Methodology Code:** Mass Spectrometry
Synthetic cannabinoids have been a vital issue to public health since the mid 2000’s as the abuse has steadily induced increasing cases of fatalities. The basis of the fatalities has been due to varying effects on numerous organs, which has made deciphering and counteracting this epidemic challenging. Acute toxic effects include tachycardia, seizures, possible suicidal tendencies and psychotic episodes occur with synthetic cannabinoid abuse not observed in marijuana use. The current project aims to introduce new insight into this toxicity of synthetic cannabinoids versus marijuana. Given that ingestion is typically via inhalation through smoking, an investigation into the pyrolysis of cannabinoids was initiated to evaluate the thermal degradation products as a cause of toxicity.

The first objective of the project was to identify the thermal degradation products. Parent cannabinoids were studied using an in-house constructed and optimized apparatus followed by GC-MS analysis, in which over 50 products were observed. Three major trends were seen and allow for predictive breakdowns of compounds not analyzed. The pyrolytics may have an impact on the toxicity, but must be shown to be absorbed by the user, which leads to the second objective of the project. Post mortem blood samples from fatality cases were obtained and are being analyzed for the presence of thermal degradation products. An adaption of a previously reported LC-MS/MS method following liquid-liquid extraction has been optimized for the parent compounds reported in each case as well as their predicted pyrolytic products. Products shown to be present in the blood will be evaluated for acute toxicity as an answer to the current mystery of the cannabinoid epidemic. This new insight into answering the cannabinoid toxicity question could have a major impact in forensic toxicology and clinical pharmacology.

This work was supported by the National Institute of Justice [2015-R2-CX-0032].

Keywords: Drugs, Forensics, Mass Spectrometry, Toxicology
Application Code: Homeland Security/Forensics
Methodology Code: Mass Spectrometry
Abuse of synthetic cannabinoids and fentanyl has led to an increase of overdoses in recent years. Sample preparation and chromatography are time consuming; simpler mass spectrometry based methods are needed to streamline drug screening. Paper spray is a rapid ambient ionization technique in which a biofluid is spotted and analyzed directly from paper with a macroscopic point without further sample preparation. However, detection limits for these compounds, which are typically in the 1-10 ng/mL range, are too high to detect toxic levels in some cases. Previous work has shown that the spray substrate impacts ion suppression and recovery, and therefore detection limits, in paper spray. Different types of paper and porous materials have been studied before to evaluate their feasibility, but these studies did not use a systematic approach that clearly identified substrate properties that impacted matrix effects. In this study, an approach was used in which spray substrates, including filter paper, chromatography paper and manufactured cellulose TLC plates, were selected that were similar with a single property significantly different. Furthermore, these substrates were subjected to modifications to try to reduce the matrix effects. Changes in ion suppression were determined by comparing the signal of the stable isotopic label (SIL) in the solvent while recovery was based on the ratio of the signal of the analyte eluted from the biofluid to the SIL. A general trend was found in that improvements to signal from improved recovery were usually paired with decreases in signal due to ion suppression. Using the properties determined in this study an ideal combination of solvent and paper was proposed for reducing the detection limits in a urine matrix.

**Keywords:** Clinical/Toxicology, Drugs, Mass Spectrometry, Paper/Pulp

**Application Code:** Clinical/Toxicology

**Methodology Code:** New Method
### Session Title
NIJ Forensic Science Research & Development Poster Session

### Abstract Title
Unearthing New Variants Related to Common Variation in Human Facial Morphology Using Genome Wide Association Study (GWAS) Methods

### Primary Author
Ryan Eller  
Indiana University - Purdue University Indianapolis

### Co-Author(s)
Andreas Wollstein, Noah Herrick, Susan Walsh

### Abstract Text
DNA Phenotyping is an up and coming area within the forensic DNA analyses community that has many possible applications, from casework to anthropological studies. While there are currently several tools available, such as HlrisPlex that aid in the prediction of categorical pigmentation phenotypes, a similar tool to predict facial structure is notably absent. If such a tool were to exist, then this may prove useful to investigators if standard genetic profiling failed to return a match to a DNA database or a ‘person of interest.’ Determining the variants associated with facial shape and their role in prediction is vital to moving facial morphology prediction in the right direction and reach the prediction levels that are currently seen for other traits. Although research is just beginning to explore common facial variation (i.e. non-disease-related variation), the use of genome-wide association studies (GWAS) to unearth variants has proved beneficial in several studies, such as Paternoster et al., 2012 and Liu et al., 2012, for quantitative variation.

Therefore, in this study we concentrated on generating both categorical facial morphology definitions as well as quantitative facial measurements. Building upon previous disease-related and some non-disease related facial morphology studies, we have generated phenotypes using 2D and 3D facial images from an admixed population of over 2500 individuals. Using a 1.7 million SNP array combined with the computationally intensive process of imputation, we were able to exponentially increase our genomic coverage allowing us to increase our statistical power for association of these phenotypic classifications. Using GWAS techniques we have assessed novel and known variants for their association and potential predictive value in common facial feature categories and quantitative measures using a US-based population set.

This work was funded by a STEM fellowship from the National Institute of Justice (2015-R2-CX-0023).

### Keywords:
Bioinformatics, Forensics, Genomics, Identification

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

### Methodology Code:
Laboratory Informatics
3D printers are becoming increasingly efficient and economical, and thus more widespread and easily accessible to consumers and the general public. Previous research has documented the release of dust particles during the printing process. However, little is known about their morphology and other characteristic features. This study was undertaken as part of a federal research grant (NIJ Grant No. 2015-DN-BX-K033) to characterize these particles so that they may be collected, recognized, and analyzed appropriately. Samples were collected from a variety of 3D printers, representing both consumer- and commercial-grade models. These printers use thermoplastic filaments, typically polylactic acid (PLA) or acrylonitrile butadiene styrene (ABS), though others may be used (nylon, polyvinyl acetate, polyurethane, etc.). Cotton or polyester-flocked swabs were used to collect dust from various surfaces within the printer chamber and surrounding areas up to 10 feet away. Particles produced from ABS filaments are most easily recognized based on color and rounded morphology via light microscopy; FTIR spectra of the particles confirmed the identification of the ABS polymer. Pigments and the ABS polymer matrix were also identified using Raman microspectroscopy. Dust from PLA printers consistently contained finer, submicron sized particles (relative to background levels) that could be observed by field emission scanning electron microscopy; however, the size of the particles precluded their specific identification as PLA. This presentation will detail the collection procedures employed to find, isolate, identify, and compare 3D printer dust particles, and a discussion of their potential applications and limitations as forensic evidence.

Keywords: Forensics, Identification, Polymers & Plastics, Trace Analysis
Application Code: Homeland Security/Forensics
Methodology Code: Microscopy
The use of nanotechnology and engineering of nanomaterials has grown exponentially in the last decade, and has found widespread application across a number of disciplines, including biology, medicine, electronics, energy, optics, and materials manufacturing, among others. These nanoparticles and other subvisible particles are present in nearly all forms of existing trace evidence, yet currently the overwhelming majority of trace examinations focus exclusively upon larger particles. In this era where highly engineered nanoscale materials are being introduced at increasing rates, it is inconceivable that such materials are not being regularly examined as forensic evidence. Practical forensic research is currently being undertaken by the authors in order to systematically develop approaches for the isolation, analysis, and interpretation of particles on the nanoscale, effectively equating the sensitivity of trace evidence to that of DNA analysis. While the smallest particles in this range may require higher resolution instrumentation, the majority of these particles can be characterized effectively by applying the suite of microanalytical methods present in most trace evidence laboratories today (stereomicroscopy, polarized light microscopy, and scanning electron microscopy). Here we present the first part of our research: describing the relevance, classifications, and applications of nanoparticles, then following with information about how these particles can best be recognized and collected in a forensic science laboratory.

Keywords: Forensics, Nanotechnology, Sample Preparation, Trace Analysis
Application Code: Homeland Security/Forensics
Methodology Code: Microscopy
Microspheres are used in an increasing variety of applications, from personal care products to food and industrial applications. Glass microspheres represent a significant subset of the microsphere market and are encountered in cosmetics, paints, plastics, building materials, and other applications. While they are used in a variety of consumer-grade products, their size, transparency, and shape can make them difficult to find or easy to overlook. For example, in solution, an isotropic, glass microspheres may be confused with an immiscible phase. Despite such difficulties, the size range (~5–1,000 µm) and composition (glass), make them accessible and potentially useful indicators of products, activity, or associations.

This poster will cover the range of physical, optical, and elemental characteristics of reference microspheres obtained from manufacturers and the ways in which glass microspheres can be located and characterized in industrial and consumer applications, e.g., cosmetics, spackle, and polymers. When present in dust, microspheres may be encountered as free particles, where they may be the sole basis of an association, or they may be encountered in a matrix, e.g., a polymer or ceramic, where they could be used to improve the significance of an association. The results from these analyses illustrate some of the ways in which microspheres can be located, characterized, and interpreted in the context of a forensic investigation.

Keywords: Characterization, Forensics, Microscopy, Nanotechnology
Application Code: Homeland Security/Forensics
Methodology Code: Microscopy
Whether we are aware of them or not, small particles abound in the environments that surround us. Small particles may be engineered for use in manufactured products, be present in dusts generated from man-made industrial processes, or occur naturally in the environment. Some of these particles are just barely visible, while others are so small that they cannot be resolved by the human eye. These subvisible and submicrometer particles (nanoparticles) offer potential as forensic evidence, but they are presently unexploited due to the challenges that their small size present.

One example of subvisible particles is the toner powder used in laser printers and copiers. Presently, most existing research on forensic toner analysis focuses on document examination, i.e., analysis of printed toner, rather than on trace evidence. However, toner is widely used, and these small particles are easily transferred and rarely noticed. Identification of trace amounts of toner, e.g., on hands or clothing or in dust, could be used to provide investigative leads or associate them with a scene and/or victim, particularly if the particles are suggestive of a specific toner.

This poster will discuss the results from an analytical study of more than 50 different toner samples. This research evaluates microscopic morphologies observed by light microscopy and scanning electron microscopy, and chemical properties determined by Raman spectroscopy, of the known toner samples, providing methods that can be used in the forensic laboratory to identify and classify toner particles. Analytical differences observed within the sample set, the prevalence of background toner particles in different environments, and limitations of this approach will be covered.

Keywords: Characterization, Forensics, Microscopy, Nanotechnology
Application Code: Homeland Security/Forensics
Methodology Code: Microscopy
The National Institute of Justice (NIJ) is the research, development and evaluation agency of the U.S. Department of Justice. NIJ’s Office of Investigative and Forensic Sciences maintains a program of external funding for R&D in forensic sciences. This program is a leading federal funder in this mission space, and the portfolio spans a broad range, from fundamental research, to development of prototype devices, to validation of novel instruments and methods.

Forensic science is a collection of applied disciplines that draws from all branches of science. Nevertheless, forensic scientists most often tend to be concerned with the detection, collection, separation, and analysis of biological and chemical samples. Because of the unique circumstances of forensic evidence, there is an ongoing need for these analyses to be done on ever smaller, degraded or mixed samples. Balancing that, is the need to ensure that analytical methods applied to these challenging samples are objective, rigorously tested, and foundationally valid. These needs drive NIJ’s continuing R&D investments in analytical chemistry and bioanalytical science. Advances in mass spectrometry, electrophoresis, applied spectroscopies, microscopy and microfluidics, among others, have yielded or show promise for forensics. NIJ anticipates continued interest in advancing technologies for forensic application. In this effort, NIJ strives to engage the analytical chemistry and applied spectroscopy research community to bring novel perspectives to solving forensic problems.

An overview of NIJ’s R&D funding programs will be presented, with the objective of introducing the Pittcon audience to the available options for research support. These range from fellowship opportunities for students, to grant funding programs for basic research with potential for long term impact on forensic science, to applied research and development focused on improving forensic practice immediately.

**Keywords:** Forensics

**Application Code:** Homeland Security/Forensics

**Methodology Code:** New Method
Enzyme engineering seeks to create an enzyme, either de novo or by modification of a known protein, with a new desired function. While biocatalysis has advantages over conventional catalytic processes, there most often are not natural enzymes with the desired properties many commercial applications. For example, many DNA polymerases can replicate DNA accurately at high speeds but are inhibited by bases damaged by environmental mutagens, oxidative stress, or UV light. Polymerases may not be able to insert nucleotides opposite a lesion or may do so with reduced accuracy. Specialized polymerases may be well suited to bypass the damage, but are typically less accurate and efficient, even on undamaged DNA. This accuracy tradeoff allows genomic replication, and life, to continue, but can also contribute to antibiotic resistance or oncogenesis. A hybrid DNA polymerase that retains the accuracy and speed of replicative polymerases, while incorporating specific lesion-bypass abilities, would be a useful biochemical tool. This research focuses on the common oxidative DNA lesion 8-oxoguanine, a small modification yet often mutagenic. The additional hydrogen bond donor allows formation of a Hoogsteen pair with adenosine when 8-oxo-dG resides in the syn conformation. To identify variants for accurate bypass, multiple criteria were used to identify positive mutations. Residue scanning was used to assess mutations’ effects on affinity towards both the preferred anti conformation and the mutagenic syn conformation. Our computational method THEMATICS was then used to filter out mutations that affect the electrostatic properties of catalytic residues and therefore are more likely to affect the polymerase activity negatively. These methods are being utilized to identify specific variants for biochemical characterization, including thermal stability, catalytic activity, lesion bypass capability, and fidelity.

Supported by NSF-MCB-1517290 and the National Institute of Justice.
Forensic face recognition attempts to identify the suspects from a huge amount gallery photos for the current probe image. The key challenges for forensic face recognition are subject to a variety of internal/external impact factors under different views, illuminations, resolutions, modalities, periods when probe images are captured in the surveillance environments without collaborations. Up to now, there is no working face recognition system that has been accepted within the judicial system. To this end, we propose to develop an effective deep structure to better handle the multiple factors within forensic data. Specifically, we build a novel Deep Robust Encoder (DRE) through locality preserving low-rank dictionary to extract robust and discriminative features from corrupted face data, where a low-rank dictionary and a regularized deep auto-encoder are jointly optimized. With the features extracted with our DRE, we adopt standard classifiers, e.g., NNC, SVM, to evaluate the face recognition performance in terms of classification accuracy. We conduct experiments on Matlab 2014 with CPU i7-3770 and 32GB memory size for a personal computer. Experimental results on several face benchmarks verify the effectiveness of our algorithm in better handling multiple factors, compared with the state-of-the-art approaches. This concludes that our proposed model could better address the multiple factors with forensic faces. Finally, this work is supported by the NIJ Graduate Research Fellowship 2016-R2-CX-0013.
The One Pot methamphetamine production method has become the primary method of choice in clandestine drug laboratories across the United States, due to its simplicity and the availability of required materials. This study was undertaken to determine the feasibility of the detection of methamphetamine clandestine laboratories through monitoring waste water effluents. Waste water samples were collected from small and large city municipalities and analyzed via solid phase extraction with liquid chromatography-tandem mass spectrometry for methamphetamine, pseudoephedrine, amphetamine, and an over-reduced product characteristic of One Pot methamphetamine synthesis, CMP [1-(1',4'-cyclohexadienyl)-2-methyl aminopropane]. A survey of urine samples (N=47, 2% CMP) that were positive for methamphetamine was conducted, and all four target compounds were similarly detected making differentiation of clandestine laboratory effluent from urinary excretion challenging. This work demonstrates the potential for analyzing waste water to detect clandestine One Pot methamphetamine laboratories and methamphetamine abuse within a community.

**Keywords:** Drugs, Forensic Chemistry, Liquid Chromatography/Mass Spectroscopy, Solid Phase Extraction

**Application Code:** Homeland Security/Forensics

**Methodology Code:** Liquid Chromatography/Mass Spectrometry
Often in missing persons cases, bone, teeth, hair, and decomposed tissue are the only samples remaining for identification. Exposure to harsh environmental conditions may also cause DNA degradation, damage, and/or inhibition, making these samples challenging to process. Human skeletal remains are often inhibited by humic acid, melanin, hematin, collagen, and calcium. Inhibitors may be co-extracted with the DNA, can interfere with PCR, and may reduce downstream DNA typing success. Current DNA identification methods include capillary electrophoresis based short tandem repeats (STRs), which are currently the gold standard. Single nucleotide polymorphisms (SNPs) are single base changes in the genome that can also be used for bio-ancestry and phenotypic information. Massively parallel sequencing (MPS) is a newer technology used in the forensic science field. It has the ability to expand our current technologies as more genetic information can be retrieved and simultaneous analysis of different (and more) markers can be incorporated (Eg. iiSNPs, STRs, aiSNPs).

An effective DNA extraction method is critical to obtain clean DNA from difficult samples. However, little is known regarding the compatibility of common DNA extraction methods with MPS chemistries. The goal of this study was to evaluate the efficiency of various DNA extraction methods to remove PCR inhibitors from skeletal remains prior to MPS. Samples were extracted using either organic or commercial kits commonly used in forensic laboratories. DNA was extracted from blood, hair, muscle, and bone after being spiked with high amounts of inhibitors. These samples were then sequenced using the Precision ID Library kit and an early access panel for degraded samples on the Ion S5™ System, and the ForenSeq™ DNA Signature Prep Kit on the MiSeq FGx™. Although the two MPS chemistries were differentially tolerant to the inhibitors tested, the results showed that all extraction methods were compatible with both MPS systems.
Since Cannabis sativa is a controlled substance in many parts of the world, the ability to track its biogeographical origin could provide law enforcement with investigative leads regarding its trade and distribution. Using autosomal, chloroplast, and mitochondrial DNA, allows not only for prediction of biogeographical origin of a plant, but also allows for genetic identification.

A previously validated 13-autosomal STR multiplex was used to genotype 496 samples. Samples were analyzed from four different sites: 21 seizures at the US-Mexico border, Brazil, hemp seeds, and Chile. In addition, a previously reported multi-locus system was modified and optimized to genotype five chloroplast and two mitochondrial markers. For this purpose, two methods were designed: a homopolymer STR pentaplex and a SNP triplex.

For autosomal typing, distinguishable profiles were generated from 381 samples that yielded full STR profiles and 44 duplicate genotypes within seizures were observed. Phylogenetic analysis and case-to-case pairwise comparisons of the 21 border seizures revealed the genetic association of nine seizures that formed a reference population.

For mitochondrial and chloroplast typing, subsampling was performed and 141 samples were genotyped. As expected, extensive haplotype sharing was observed; five distinguishable haplotypes were detected. Haplotype sharing was observed between the US border seizures, Brazil, and Chile while the hemp samples generated a distinct haplotype.

Results revealed that both autosomal and lineage markers could discern population sub-structure. Phylogenetic analysis of the four populations using neighbor joining were estimated with the GDA software. Parsimony analysis was then performed with the PAUP* software. The STRUCTURE software was employed to investigate the population structure among groups. And finally, the R package, Adegenet, was used to visualize the genetic distance of the populations using Principal Component Analysis (PCA).

Keywords: Capillary Electrophoresis, Database, Forensics, Nucleic Acids
Application Code: Genomics, Proteomics and Other 'Omsics
Methodology Code: Capillary Electrophoresis
Abstract Text

In many cases, probative information may be gained from evidence through the identification of the source body fluid in addition to identification of a person. While serological tests are commonly used by crime laboratories, they are generally presumptive in nature due to differing levels of specificity and sensitivity. However, nucleic-acid based methods have been proposed as a means to provide a more confirmatory method of body fluid identification (BFID). These methods allow for the identification of a greater number of body fluids as well as the ability to co-analyze with DNA and consume less evidentiary sample. Recently, miRNAs have been suggested as a biomarker for BFID due to their small size (19-22 nucleotides), making them ideal for analyzing highly degraded samples.

In this study, we generated both DNA and miRNA profiles from single co-extracted samples using capillary electrophoresis-based methods. For the miRNA analysis, we expanded on a previously reported linear primer system in order to include additional markers. In this panel, an 8-marker system was designed to differentiate venous blood (miR-451 and miR-142-3), menstrual blood (miR-141-3 and miR-412), semen (miR-891 and miR-10), and saliva (miR-205). In addition, an endogenous reference gene (let-7g) was included to confirm successful reverse transcription and amplification.

Each primer set was evaluated in singleplex to assess cross-reactivity between body fluids and genomic DNA as well as to determine optimal amplification conditions. All samples tested yielded full STR profiles from the DNA fraction and let-7g amplification from the RNA fraction. Although some cross-reactivity was observed, a presence/absence scheme was developed to distinguish between venous blood, menstrual blood, semen, and saliva.

Keywords: Capillary Electrophoresis, Forensics, Nucleic Acids
Application Code: Genomics, Proteomics and Other 'Omics
Methodology Code: Capillary Electrophoresis
Abstract Text

The chemical analysis of forensic traces often results in complex, high-dimensional data. The statistical inference of the source of these traces requires assigning probability distributions to the data. Chemometric techniques often involve data reduction and machine learning such as principal component analysis, neural networks, or support vector machines. However, while these are useful for discrimination and classification, they are difficult to use in the probabilistic framework necessary to properly quantify the weight of forensic evidence.

One approach proposes to circumvent this problem by reducing the data dimension by assigning pairwise similarity scores between i.i.d. objects that compose the evidence (in an analogous manner to support vector machines) and to build probabilistic models based on the univariate distribution of scores, disregarding the dependency between multiple scores calculated pairs of objects which reuses a common object.

In this presentation we will summarize recent advances made on kernel-based models to propose a probabilistic and multiclass version of SVM. Our model captures the dependencies between pairwise scores from a hierarchical sample and models them in the kernel space using a linear model. Our model is flexible to accommodate any kernel satisfying basic conditions and as a result is applicable to any type of complex high-dimensional data. An important result of this work is the asymptotic multivariate normality of the scores as the data dimension increases. As a result, we can: 1) model very high-dimensional data when other methods fail; 2) determine the source of multiple samples from a single trace in one calculation. We will provide examples of real-life problems using data from very small particles and dust analyzed by SEM/EDX, and colors of cotton fibers quantified by microspectrophotometry.

Keywords: Forensics, Pattern Recognition, Quantitative, Statistical Data Analysis

Application Code: Homeland Security/Forensics

Methodology Code: Data Analysis and Manipulation
Rapid and efficient processing of sexual assault evidence is an urgent need in forensic DNA analysis to accelerate forensic investigation and reduce casework backlogs. One of the major challenges in the processing of sexual assault cases is to differentially extract evidence samples, separation of the victim’s cells (epithelial) from the perpetrators cells (sperm). Current methodologies and techniques consist of multiple time consuming steps, including selective cell lysis, centrifugation and differential DNA separation. However, there is no integrated rapid sperm isolation and differential extraction platform which includes cell isolation and quantitation, in preparation for downstream genomic analyses.

In this abstract, we present a microfluidic platform technology to specifically capture sperm cells and differentially extract male DNA in complex forensic samples. To capture sperm, the microfluidic chips are modified with a unique oligosaccharide molecule, which is located on the extracellular matrix (i.e., zona pellucida (ZP)) of the oocyte to denote a ligand for human sperm-oocyte binding. Our platform isolates sperm with >90% of capture efficiency, and removes up to ~93% of epithelial cells from heterogeneous cell populations. Captured sperm are then lysed on-chip for potential downstream genomic analyses. In collaboration with the Broward County Sheriff’s Office, we have also presented that these microchips can selectively and efficiently capture sperm from mock sexual assault samples in a cost-effective manner. This next generation differential extraction process considerably reduced assay-time to 80 minutes, providing an inexpensive alternative to multi-step, labor-intensive differential extraction, thus potentially accelerating identification of suspects; advancing public safety.
Particle combination analysis using very small particles (VSP) is a new approach, highly significant for its potential to expand the number of cases to which trace evidence can meaningfully contribute and for its ability to include a quantitative statistical approach to data interpretation. The laboratory analyses are highly efficient, utilizing existing crime laboratory personnel and equipment.

Prior research, employed reasonable choices of analytical and statistical parameters which were sufficient to demonstrate feasibility and potential. Systematic development and validation of these methods requires that the analytical and statistical parameters be more critically examined, and that the key factors influencing the performance of the methods be identified.

Determination of the key factors and the magnitude of their effects will result in a significantly improved capability and provide necessary input to experimental designs that will permit systematic improvement and optimization. This will enable transition of particle combination analysis to practice and contribute to the fundamental advancement of a new quantitative and broadly applicable approach to trace evidence. Well-documented factors and effects for one VSP analysis protocol will allow parallel, collaborative assessments of alternative options for high efficiency analysis of VSP (such as micro Raman methods, microXRF, genetic analysis, or alternative SEM/EDS protocols).

This project was supported in part by Award Nos. 2012-DN-BX-K041 and 2015-DN-BX-K046 awarded by the National Institute of Justice, Office of Justice Programs, U.S. Department of Justice. The opinions, findings, and conclusions or recommendations expressed in this presentation are those of the authors and do not necessarily reflect those of the Department of Justice.

Keywords: Elemental Analysis, Forensics, Microscopy
Application Code: Homeland Security/Forensics
Methodology Code: Microscopy
Detecting and quantifying cocaine in oral fluid is of significant importance for practical forensics. Up to date, mainly destructive methods or biochemical tests have been used, while spectroscopic methods were only applied to pretreated samples. In this work, the possibility of using resonance Raman spectroscopy to detect cocaine in oral fluid without pretreating samples was tested. It was found that ultraviolet resonance Raman spectroscopy with 239-nm excitation allows for the detection cocaine in oral fluid at 10 \([\text{micro}]\text{g/mL}\) level. Further method development will be needed for reaching the practically useful levels of cocaine detection.
Raman spectroscopic mapping has become more and more popular as an analytical method. One drawback of Raman microspectroscopic analysis is interference from substrates. Our lab has evaluated several approaches to overcome this problem targeting the identification of body fluid traces for forensic purposes. First, we varied the excitation wavelength, but no one wavelength worked well for all the common substrates. Using different excitation wavelength for different substrates was impractical. Then background subtraction was attempted and works very well for homogeneous substrates, but not for heterogeneous ones. Here we report on a new universal approach based on Raman hyperspectroscopy and MCR data analysis. The program was used for substrates that are fluorescent and heterogeneous and was able to extract the body fluid signal in all cases.

This project was supported by Award No. 2014-DN-BX-K016 awarded by the National Institute of Justice, Office of Justice Programs, U.S. Department of Justice (I.K.L.). The opinions, findings, and conclusions or recommendations expressed in this publication are those of the authors and do not necessarily reflect those of the U.S. Department of Justice.

**Keywords:** Chemometrics, Forensics, Raman Spectroscopy

**Application Code:** Homeland Security/Forensics

**Methodology Code:** Chemometrics
The United Nations Office on Drugs and Crime has identified 20 plant species that are increasingly being used as “legal highs” (i.e. psychoactive products that remain unscheduled). Their legal status shields users and traffickers of these products from prosecution. Although the bulk of these materials are not cultivated in Europe or the United States, they are imported from various regions of the world including Asia, Africa and South America, and are thus readily available. A major challenge to the legislation of the use and sale of these substances is that it is extremely difficult to distinguish them from innocuous plant-based products such as herbs, spices, and foods. The ability to do so would be especially useful for Border Protection Agents, so that commercial cargo can be screened for their presence. We sought to address this issue by developing a method that would enable detection of diagnostic small-molecule chemical signatures indicative of the presence of plant-based legal highs. In this approach, the headspace volatiles of “legal high” plant materials are concentrated on polydimethylsiloxane (PDMS) SPME fibers, which are subsequently analyzed by direct analysis in real time-high resolution mass spectrometry (DART-HRMS). The observed chemical signatures are then subjected to multivariate statistical analysis approaches to enable classification and identification of the plant material. Kernel discriminant analysis (KDA) of the DART-MS data showed that the headspace signature could be used to accurately identify the bulk material. External validation was also performed to assess the reliability of the technique and was 100% accurate in all tests. These results demonstrate proof-of-concept for the creation of a database against which cargo-container derived headspace can be screened for the detection and identification of plant-based legal highs.

This work was supported in part by the United States National Institute of Justice (grant 2015-DN-BX-K057).
A Validated and Rapid Method for the Quantification of Psychoactive Materials in Complex Plant Matrices Using Direct Analysis in Real Time-High Resolution Mass Spectrometry

The quantification of natural products in complex matrices is a common practice in a number of fields, including medicine and forensics, among others. When the analyte of interest is contained within a complex plant matrix, the steps towards its quantification are far from straightforward. Our objective was to establish a procedure for the quantification of psychoactive materials found in plants being abused as “legal highs”, using an ambient ionization mass spectrometry technique that would greatly reduce the sample preparation typically required using traditional methods. Specifically, we describe an FDA validated method for the quantification of psychoactive atropine in [i]Datura[/i] spp. seeds using direct analysis in real time-mass spectrometry. Calibration curves for atropine in a 1:1 v/v ethanol/water solvent using atropine-D3 as an internal standard were obtained over a linear range of 0.49 to 500 ppm. The limits of detection and quantification were determined to be 0.49 ppm and 0.98 ppm, respectively. [i]D. stramonium[/i], [i]D. ferox[/i] and [i]D. inoxia[/i] seeds were extracted and analyzed. Average concentrations of 15.32 +/- 0.15 ppm of atropine in the [i]D. stramonium[/i] extract and 1.07 mg/g per seed were determined. The results compared well with reported levels determined using traditional approaches. Atropine in seed extracts of [i]D. inoxia[/i] and [i]D. ferox[/i] fell below the lower limit of quantification, but the extrapolated concentrations of atropine in their extracts were 0.83 +/- 0.14 ppm and 0.76 +/- 0.05 ppm, respectively. The results indicate that DART-MS can be used as a rapid means to quantify atropine in plant samples while avoiding significant sample preparation steps. Furthermore, the method can be applied to the quantification of other biomarkers in plant materials, despite the complexity of the plant matrix.

This work was supported by the National Institute of Justice (grant 2015-DN-BX-K057).

Keywords: Forensics, Forensic Chemistry, Mass Spectrometry, Quantitative
Application Code: Homeland Security/Forensics
Methodology Code: Mass Spectrometry
For years, fingerprint samples have been believed to be useful only for matching purposes using the unique ridges, shapes, and sizes. The traditional pictorial comparison used for the past century has been useful for the identification of some individuals, but many fingerprint samples have been labeled “unusable” due to smudging, smearing, or any one of a myriad of reasons that could cause inconclusive matches. These samples can, however, still be of use. The chemical composition contains sebum, sweat, and a variety of lipids found to be secreted from the fingertips. The methods our group has developed use the amino acid content in fingerprints to differentiate between male and female fingerprints—one of the many possible characteristics that can be identified.

The research described here further explores the concept of determining attributes of an originator via the fingerprint contents mentioned above. Currently, research has focused on utilizing amino acids with known cascades specifically to see if decreasing the number of amino acid targets would interfere with the ability to determine if the sample is from a male originator or a female originator. This previously studied physical trait was chosen both to prove the viability of a single analyte assay for fingerprint analysis and to provide corroboration for the data obtained by the multi-analyte assay from our previous work. Additionally, we have explored the development of a paper-based strip for the colorimetric detection of metabolites.

The systems presented here are designed to be versatile and adjustable enzyme cascades that will produce easily interpretable results. The successful development of this concept would lead to a new treatment of fingerprints as a source of evidence. This project could revolutionize on-site forensic analysis, as a result, accelerating the rate of criminal investigations.

Keywords: Amino Acids, Bioanalytical, Forensic Chemistry, Sensors
Application Code: Homeland Security/Forensics
Methodology Code: UV/VIS


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<td>Primary Author</td>
<td>Crystal Huynh</td>
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<tr>
<td>Author</td>
<td>University at Albany, SUNY</td>
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<td>Co-Author(s)</td>
<td>Erica K. Brunelle, Jan Halamek, Lenka Halamkova</td>
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Abstract Text

Fingerprint analysis refers to the process of comparing fingerprint patterns by an expert and/or an automated fingerprint identification system. This method has become a universally accepted method for identification. This method of pictorial comparison is also one of the few forensic areas that have yet to see improvement in the past years, even with the recent increase of interest in forensics. Currently, the analysis ends with this matching methodology causing the field to be dependent on the presence of a stored matching print or a matching print from an individual that is physically present. Due to this limitation, many analyses result in inconclusive outcomes. What is often overlooked is that those latent prints are created by sweat and sebum emulsions excreted by the fingertips. Those emulsions have their own unique chemical compositions for each individual making them possible biological samples for analysis. Our lab has developed a bioaffinity-based cascade for the determination of biological sexes from the chemical composition of the sweat/sebum left as the latent prints.

The research presented here addresses the current limitations in fingerprint analysis using a bioassay system that focuses on the components of fingerprints. Bioaffinity-based assays have been developed for the determination of biological sexes from those components. In one assay, L-amino acid oxidase was used to target the amino acids present in the sebum and sweat left on latent fingerprints. Further research has led to the testing of authentic fingerprint samples collected from various surfaces as well as the development of other bioaffinity-based assays capable of differentiating between biological sexes via less complex systems. Other bioaffinity-based assays will also be developed in the future for the determination of other physical attributes such as age group and ethnicity.

Keywords: Amino Acids, Bioanalytical, Forensic Chemistry
Application Code: Homeland Security/Forensics
Methodology Code: UV/VIS
Establishing Exposure to Plant-Based Psychoactive Materials by Mapping Diagnostic Biomarkers in Fingerprints—A MALDI Mass Spectrometry Imaging Study

One approach to evading prosecution for illicit drug use is to utilize currently unscheduled substances such as psychoactive plants. Barriers to legislating the use of such substances include the absence of standard protocols for their identification and the non-existence of methods that establish a connection between the abuser and the substance abused. To address this, our objective was to develop a technique by which evidence of contact with these mind-altering substances could be linked to an individual, as this would provide valuable information to law enforcement and healthcare practitioners alike. We demonstrate here that prior handling of plant-based psychoactive materials can be established through detection of diagnostic biomarkers by SpiralTOF matrix-assisted laser desorption ionization high-resolution mass spectrometry imaging. Furthermore, the compounds remain detectable in the fingerprint even after the print has aged. Plant products representing several relevant species were handled by rubbing the material between the fingers, whereafter fingerprints were deposited and prepared for analysis by SpiralTOF MALDI MS. Ion images of selected \( m/z \) values showed the spatial distributions of diagnostic small molecules indicative of exposure to the psychoactive plant materials. These included dimethyltryptamine from \( Mimosa hostilis \) and \( Psychotria viridis \), and harmala alkaloids derived from \( Peganum harmala \), as well as others. Importantly, the observed images were identical to those generated using endogenous lipids such as oleic acid. Plant biomarkers in fingerprints remained detectable for at least one week after being deposited. The findings illustrate that handling of psychoactive plant material by an individual can be definitively established solely through visualization of fingerprint images based on plant biomarkers.

This work was supported by the United States National Institute of Justice (grant 2015-DN-BX-K057).

Keywords: Forensics, Imaging, Laser Desorption, Mass Spectrometry
Application Code: Homeland Security/Forensics
Methodology Code: Mass Spectrometry
In this proof-of-concept study, a novel method for the detection and identification of organic gunshot residue (OGSR) was developed. This method consisted of a two-step process: the first step utilized highly sensitive fluorescence hyperspectroscopy to image a selected area and detect particles with specific size and emission properties. The particles of interest were then characterized with Raman spectroscopy to confirm their OGSR origin. This process was undertaken using 9 mm caliber gunshot residue particles on adhesive tape substrates. Two samples were investigated: one having a known number of OGSR particles and the other possessing an unknown number of particles. Investigation of these two samples demonstrated that the developed method is effective for the detection and identification of OGSR particles. Meanwhile, debris and other artifacts were successfully identified as non-GSR particles. This method presents a potential means for forensic analysts to screen for the presence of OGSR particles. This “double-screening” of the samples—by first using fluorescence and subsequently utilizing Raman spectroscopy in order to investigate the samples—provides a twofold method that allows for the accurate and effective detection and identification of OGSR particles.

This project was supported by Award No. 2016-DN-BX-0166 awarded by the National Institute of Justice, Office of Justice Programs, U.S. Department of Justice (I.K.L.). The opinions, findings, and conclusions or recommendations expressed in this publication are those of the authors and do not necessarily reflect those of the U.S. Department of Justice.
Identification of Species’ Blood by Attenuated Total Reflection (ATR) Fourier Transform Infrared (FT-IR) Spectroscopy

Blood is one of the most common and informative types of biological evidence found at a crime scene. In forensic investigations it is crucial to identify the origin of a blood stain. However, current standard methods employed for the analysis of blood samples are destructive and time-consuming.

In this study, attenuated total reflection (ATR) Fourier transform-infrared (FT-IR) spectroscopy was used as a confirmatory, nondestructive, and rapid method for identifying species based on blood. Bearing in mind forensic purposes, differentiation of human and nonhuman blood samples was targeted, and partial least squares discriminant analysis (PLSDA) model demonstrated complete separation between human and animal donors. The models also distinguished between three separate species, namely human, cat, and dog. The method was validated in two external manners: using unknown samples outside of the dataset from human, cat, and dog blood, as well as samples which were more different than any in the training dataset (of different species, breeds, and genders). Classification predictions of unknown blood donors performed by the model resulted in 100% accuracy. This study demonstrates ATR FT-IR spectroscopy’s great potential for blood stain analysis and species discrimination. Furthermore, the commercial availability of portable ATR FT-IR instruments affirms the potential for the implementation of such blood stain analyses both at a crime scene as well as in the lab.

This project was supported by Awards No. 2011-DN-BX-K551 and 2014-DN-BX-K016 awarded by the National Institute of Justice, Office of Justice Programs, USA, Department of Justice (I.K.L.).

Keywords: Biological Samples, Chemometrics, Forensics, FTIR
Application Code: Homeland Security/Forensics
Methodology Code: Vibrational Spectroscopy
Bearing in mind forensic purposes, a nondestructive and rapid method was developed for race differentiation of peripheral blood donors. Blood is an extremely valuable form of evidence in forensic investigations so proper analysis is critical. Because potentially miniscule amounts of blood traces can be found at a crime scene, the ideal method is nondestructive while providing substantial information about the sample. In this study Raman spectroscopy was applied with advanced statistical analysis to discriminate between Caucasian and African American donors based on dried peripheral blood traces. Spectra were collected from 20 donors varying in sex and age. Support vector machines discriminant analysis (SVMDA) was used for differentiation between the two races. An outer loop subject-wise cross-validation (CV) method served to evaluate the performance of the SVM classifier for each individual donor from the training data set. The performance of SVMDA, evaluated by the area under the curve (AUC) metric, showed 83% probability of correct classification for both races, and a specificity and sensitivity of 80%. This preliminary study shows promise for distinguishing between different race donors of human blood. The method provides rapid and reliable results without any preparation, destruction, or consumption of the sample—thus making it an ideal method for real life crime scenes.

This project was supported by Awards No. 2011-DN-BX-K551 and 2014-DN-BX-K016 awarded by the National Institute of Justice, Office of Justice Programs, U.S. Department of Justice (I.K.L.).

Keywords: Biological Samples, Chemometrics, Forensics, Raman Spectroscopy
Application Code: Homeland Security/Forensics
Methodology Code: Vibrational Spectroscopy
Designing a Methodology for Body Fluid Identification with a Portable Raman Spectrometer

Identification and preservation of body fluid traces is a crucial step in forensic science. These traces are potential sources of DNA evidence, which can be instrumental in making convictions in criminal cases. This step is also very difficult, due to the specificity, imprecision, and destructive nature of current investigative tests. Our laboratory has recently developed a non-destructive, universal method of identifying body fluid traces using a desktop Raman spectrometer. However, it is important that trace evidence be analyzed and identified in situ, and immobile desktop instruments are unable to meet this need. This has created a need for portable instruments for use in the field. We have worked on translating this methodology for larger spectrometers to a portable instrument. We have designed a specialized tool for sampling with this instrument, thus making the methodology more specific. We also focused on assessing the instrument’s ability to differentiate between body fluids. Spectra of several body fluid were acquired and run against verified models of identification for larger Raman spectrometers.

Keywords: Forensics, Portable Instruments, Raman Spectroscopy, Trace Analysis
Application Code: Homeland Security/Forensics
Methodology Code: Portable Instruments
**Session Title**: NIJ Forensic Science Research & Development Poster Session

**Abstract Title**: Wearable Interbody Communication Sensors for Body Area Networks

**Primary Author**: Ahmed Eltawil  
University of California, Irvine

**Co-Author(s)**: Ahmed Khorshid

**Abstract Text**

Wearable devices are rapidly being adopted as means of improving health care services. However, most wearable platforms are limited to a single point of contact location with the human body due to area and power consumption restrictions, dictated by the wireless interface. An emerging technology that holds the potential for solving such issues is Intra-body Communication (IBC), where the signal is harmlessly confined to the human skin rather than propagated in the air. Using this approach, multiple sensors, distributed on the human body, can intercommunicate without the need for an air interface, leading to ultra-compact, precise, low-power sensors. A single wireless hub, can connect to distributed IBC sensors to serve as the gateway to the external world using traditional air interfaces (e.g. Bluetooth etc.). Distributing such small, low-power, smart sensors in a well studied pattern over the human body will allow users to monitor vital signals, such as body temperature and electrocardiogram with unprecedented accuracy. Such data can then be analyzed for training or operational safety purposes where stress profiles and reactions can be accurately identified.

One of the initial research goals of the project is to understand and compare the main potential data carriers for IBC; namely using electro-magnetic waves, ultrasonic waves and magnetic coupling. Pros and cons of each approach are studied and the frequency response of different body tissues to these modalities are quantitively documented. Based on these studies, a model for intra-body communications, using electromagnetic galvanic coupling has been developed and adopted as the appropriate data carrier for this emerging technology. In the proposed model, biological parameters of the human body are accurately modeled, as well as assumed to be variable, taking into consideration the impact of important factors; such as age and weight, on these parameters and thus on the overall system profile.

**Keywords**: Bioanalytical, Biomedical, Biosensors, Sensors  
**Application Code**: Biomedical  
**Methodology Code**: Sensors
Heat Dissipating Strategies in Pyrophytic Plant Follicles

Existing structural materials are thick, rigid, heavy, and cumbersome for use as protective clothing, necessitating the development of lightweight, thin and flexible materials that shield from high-velocity projectiles and overheating. Nature, over millions of years of evolution, has designed hierarchically structured bio-composites with superlative damage tolerance capabilities. Oftentimes, these materials feature multi-functional capabilities, such as thermal robustness coupled with mechanical resistance. One such multifunctional bio-composite is found in pyrophytic plants, that have evolved fire-resistant follicle valves to protect their encapsulated seeds. In addition, these valves also protect seeds from pecking and abrading predators. The follicle valves are made of organic polymeric components and yet exhibit remarkable thermal and mechanical tolerance, due to their microstructure and chemical components. The follicle valves consist of three distinct layers - a dense lignified exocuticle called the exocarp, a thick-walled cellulosic fibrous mesocarp, and an endocarp consisting of thin-walled cellulose fibers. Through thermal analyses, we determined that the exocarp shows significantly higher circumferential thermal conductivity when compared to the through-thickness direction, indicating that it possibly functions as a heat-dissipating shield. In addition, heating the follicle valves in air to ~200°C revealed an expansion of the exocarp, called intumescence. This creates localized insulating pockets which could further inhibit flame penetration. Upon heating the follicle valves to ~600°C, the plant organics carbonize to form a dense graphitic phase – Carbon-8, a known insulator, resulting in yet another flame retardating mechanism. Design strategies from these investigations will be used as guidelines to develop next-generation multifunctional materials for NIJ applications with thermally resistant synthetic polymers like polyimides.

Abstract Text

Abstract Title: Heat Dissipating Strategies in Pyrophytic Plant Follicles

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Keywords: Biological Samples, High Temperature, Materials Characterization, Thermal Analysis
Application Code: Material Science
Methodology Code: Thermal Analysis
Each of our bodies is covered in billions of microbial cells, which we shed on objects that we touch. Recent work on the built environment (human-made structures) has highlighted the ubiquity of human microbiome signatures in these human dominated ecosystems. Previous work has demonstrated that the transfer of skin microbes to surfaces can associate objects with individual people, and that the microbial signatures are generally stable within a person, raising the potential that these microbial fingerprints could provide important physical evidence. However, a knowledge gap exists about whether skin microbes transfer to different material types and whether they persist over timescales relevant to forensic investigations. Here we investigate the effect of surface type (wood, plastic, metal, glass, and ceramic tiles) on the ability of skin microbes to transfer to an object. By applying machine learning methods using a Random Forests classifier, we discovered that plastic and ceramic surfaces were most accurate for classifying the correct participant, followed by glass and metal. We also determined that skin microbial signatures persisted on ceramic and plastic surfaces for at least one day, and became less accurate over time. Overall, we find that microbiome trace evidence samples can be tracked back to individuals with high accuracy, and can be used to narrow pools of suspects even when multiple people have touched a surface and even when the reference microbiome was collected a year ago. We conclude that skin microbes are uniquely positioned to augment friction ridge comparison when sufficient ridge detail is not available to make a positive identification. Thus, the potential for microorganisms to reveal whether a particular person has touched an object is substantial.
### Abstract Text

Suspects in sexual assault cases are commonly identified by analyzing seminal fluid that remains at the crime scene for the DNA profile. However, the use of condoms in such crimes has been on the rise, preventing the deposition of biological fluid. As a result, sexual assault evidence should also include the analysis of condom lubricants to support the current analysis that is being conducted. However, to ensure that this type of analysis is beneficial for sexual assault investigations, an important consideration is the risk of false positives from the inherent residues of personal hygiene products (PHPs) found on the skin of the victim or at the crime scene. Therefore, the aim of this study is to present a classification scheme to mitigate the predicament of misidentifying unknown samples.

In this study, 32 samples including 12 personal lubricants, 10 condoms, and 10 PHPs, were analyzed using DART-TOFMS and GC-MS. The results were statistically treated using hierarchical cluster analysis, principal component analysis, and linear discriminant analysis. The statistical classifications from both DART-TOFMS and GC-MS were compared to evaluate the advantages and disadvantages of each analytical method to differentiation sexual lubricants from PHPs in sexual assault evidence.

The presentation will disclose a classification scheme to differentiate sexual lubricants from PHPs using two instrumental methods. Additionally, the use of statistics in providing accurate classification and discrimination will be discussed. The attendees will learn how unknown samples can be classified into sample groups and how instrumental methods for sexual assault evidence can be evaluated and compared.

**Keywords:** Chemometrics, Forensic Chemistry, GC-MS, Time of Flight MS

**Application Code:** Homeland Security/Forensics

**Methodology Code:** Mass Spectrometry
Identification and Quantification of Sexual Lubricant Degradation Pathways from Exposure to the Vaginal Bacterial Environment

Due to the use of DNA analysis for identification, an increasing number of offenders are using condoms to mask their identity from law enforcement. During a sexual assault, lubricant from a condom can be transferred to the victim. In forensic lubricant analysis, the major components of condom lubricants, such as polyethylene glycol (PEG) and 1-octylamine, are used as indicators of the presence of sexual lubricants.

Bacteria natural to the vaginal cavity, including members of the Pseudomonas genus, help maintain a healthy environment. Metabolic processes of these bacterial strains can use residual lubricant that remains in or near the vagina as a possible energy source thus leading to sample degradation of the lubricant. The degradation caused by microbial exposure makes it necessary to understand how the microbes change the condom lubricant components and the overall chemical profile of the lubricant.

The degradation of two common condom lubricant components, PEG and 1-octylamine, was studied using a common vaginal microbe, Pseudomonas putida (P. putida). Toxicity tests were conducted to determine the viability of P. putida in the presence of PEG and 1-octylamine. Growth was positive at concentrations ranging from 25 ppm to 100 ppm, in the presence of both components. Subsequently, P. putida was inoculated with PEG and 8 samples were collected in triplicate over 36 hours. Lubricant degradation studies were conducted and demonstrated that P. putida might be using the PEG as a possible carbon source. Chemical degradation was measured using direct analysis in real time-time-of-flight mass spectrometry and gas chromatography-mass spectrometry. Instrumental data was evaluated and analyzed using chemometric methods.

Further understanding the interactions of bacteria found in the vaginal cavity and condom lubricants can provide forensic science and sexual assault investigation communities with a new analytical timeline for vaginal samples collected after a sexual assault.

Keywords: Bioanalytical, Forensics, Mass Spectrometry, Trace Analysis
Application Code: Homeland Security/Forensics
Methodology Code: Mass Spectrometry
Unfortunately, sexual assaults are a reality in today’s society. Increasing use of condoms reduces potential of recovering DNA evidence, and a novel approach for the analysis of other trace evidence is required. The characterization and classification of lubricants is a relatively new approach for analyzing unknown trace evidence that could be collected from the crime scene or the victim.

In this study, 20 samples from different sexual lubricant manufacturing types were tested: water-based, silicone-based, oil-based, and organic/edible lubricants, and personal hygiene products which could also be used in sexual assaults. Instrumental methods were developed for direct analysis in real time-time of flight mass spectrometry (DART-TOFMS), gas chromatography-mass spectrometry (GC-MS) and Fourier transform infrared spectroscopy (FTIR). Analytical protocols were designed to increase the identification of unique components in these lubricants to develop a classification scheme for unknown samples.

Neat lubricants, as well as solvent extracts, were analyzed in both positive and negative ionization modes using DART-TOFMS in replicates of five. Neat lubricants and extracts were also analyzed via FTIR in triplicate, and extracts were analyzed by GC-MS in triplicate. Multivariate statistical techniques were used to identify unique markers that describe each class within the larger dataset. Classification schemes were developed for each instrument individually.

The outcomes of the classification schemes are expected to separate the different manufacturing types into groups, and sub-classes within each manufacturing type. The classification schemes developed from this preliminary study will affect the forensic trace evidence community by aiding in future exploitation of evidence found at a crime scene based on the data, thus providing investigative leads and innovative techniques in the analysis of trace evidence.
The cyanoacrylate fuming method (CFM) is a widespread chemical process used in forensics to reveal latent prints on surfaces via the anionic polymerization of ethyl cyanoacrylate (ECA). The ECA monomer reacts with biological components, such as the amino acids found in sweat, which serve as initiators. Empirical studies have shown the polymerization of ECA at room temperature produces the most polymer when the relative humidity is around 80 percent. Previous studies in the Dadmun group have also shown that the anionic polymerization of ECA at low temperatures produces a larger quantity of high molecular weight polymer. Although the exclusive effects of relative humidity and temperature on the growth of poly(ethyl cyanoacrylate) (PECA) from deposited prints during fuming have been thoroughly investigated, our research exceeds the scope of current knowledge by evaluating the collaborative influence these parameters have on the method as well as how these parameters affect the characteristics of the resultant PECA polymer. To test the combined effects of humidity and temperature on the efficiency of the CFM, latent fingerprints on glass slides were placed in a fuming tank and fumed at different humidity levels and surface temperature settings. The amount of polymer accumulation and quality of the prints were compared. Our data findings suggest although there is more PECA observed at higher relative humidity, lower relative humidity provides more high molecular weight polymer, which is more ideal for the quality of the polymer and visual detail of the prints. This holistic study provides forensic scientists with more optimum procedures to obtain fingerprints from surfaces without deteriorating the evidence in the process.

This research is funded by the National Institute of Justice Grant: 2015-IJ-CX-K015.

Keywords: Forensics, Forensic Chemistry, Polymers & Plastics, Separation Sciences
Application Code: Homeland Security/Forensics
Methodology Code: Physical Measurements
Evidence Recorded in Fingernails: Oxygen and Strontium Isotopes Reveal Travel Histories

Stable isotope (SI) analysis of keratin tissues have been used to reconstruct geographic location across multiple disciplines. Drinking water contributes to the oxygen isotope ($^{18}$O) signal that reflects geographic locations, while strontium isotope ratios ($^{87}$Sr/$^{86}$Sr) come from environmental sources and relate to geologic formations. These SI combinations form unique signatures that can be used to understand an individual's travel history. This study focuses on the SI patterns of fingernail clippings from residents of the Salt Lake City, Utah region (SLC) that traveled to locations outside of the USA before returning home (NIJ STEM Graduate Fellowship, 2014-DN-BX-0003). We hypothesized that the $^{18}$O and $^{87}$Sr/$^{86}$Sr would change, as precipitation SI values and geologic formations differed between their region of travel and SLC.

The $^{18}$O values were consistent with reported travel histories. SI values were similar to SLC residents when the volunteers resided in the area and as they moved to their new locations they diverged. In contrast, $^{87}$Sr/$^{86}$Sr of the fingernail clippings displayed different patterns. We found that the $^{87}$Sr/$^{86}$Sr reflected the location of where the volunteer clipped their nails, resulting in an unexpected pattern that was attributed to how the isotopes are incorporated into keratin protein. $^{18}$O are incorporated during the protein formation and once formed the isotope signature does not change. Our findings suggest that $^{87}$Sr/$^{86}$Sr are incorporated into the fingernail keratin through environmental or bathing waters and reflect an individual's most recent location.

As one of the first multi-isotope studies on human fingernails, we report on new travel-related isotope signals that can be obtained from $^{18}$O and $^{87}$Sr/$^{86}$Sr measurements. The study has also allowed us to look into greater detail at isotope incorporation into fingernails and how these values can vary among individuals.

Keywords: Biological Samples, Forensics, Isotope Ratio MS
Application Code: Homeland Security/Forensics
Methodology Code: Mass Spectrometry
Rapid screening of narcotics and explosives is critical for ensuring public safety and controlling crime, especially at the state and local levels of law enforcement. Colorimetric reactions have been extensively used for on-site explosives and narcotics analysis as a rapid, user-friendly, and inexpensive detection platform. Although current colorimetric field methods enable on-site testing, such methods rely solely on subjective interpretation of color with a variety of operational issues including poor training, differences in color interpretation, varied chemical response due to improper mass of sample, all with no multiplexing capability. For an enhanced system to be a major improvement, it must be fully automated to reduce subjectivity, inexpensive, handheld, capable of rapid screening and parallel processing, and include an on-board optical detector. Here, we describe a centrifugal microfluidic system that accepts single-use, disposable microchips that, with embedded reagents, costs <$1 and are compatible with a modified Sony Discman® to drive fluid flow and chemical reaction. An integrated Android cellphone functions as the colorimetric detector with a custom-built 'app' for interpreting the average pixel color density and associating it with a specific narcotic or explosive. This prototype system (microdevice, instrument and smartphone) was used for multiplexed testing for the presence of various explosive and narcotics material from a single input, such as cocaine, methamphetamine, TNT, and ammonium nitrate. Color analysis was used to determine quantitative hue values to associate with positive results for each analyte of interest. These threshold values were then applied to the custom-built cellphone app for user-friendly analysis.
MicroRNAs (miRNAs) are small non-coding RNAs 18-25 nucleotides in length that have been evaluated as potential markers for the identification of forensically relevant body fluids. Due to their short length and high resistance to degradation, they provide potential for robust detection in degraded samples. High-throughput sequencing (HTS) of eight forensically relevant biological fluids was used to identify miRNAs with tissue-specific expression. Candidate miRNAs were developed and the expression patterns were assessed, identifying a panel of miRNAs plus two endogenous reference miRNAs that allow for normalization of expression without evaluation of the RNA or known input quantity. This panel uses expression detection through reverse-transcription quantitative PCR (RT-qPCR) to differentiate feces, urine, blood, menstrual secretions, and saliva. Candidates for vaginal secretions and perspiration did not readily prove to be reliable, so additional screening for distinguishing miRNAs was conducted. Biological fluid identification was found to be reliable across population samples of mixed ages, ethnicities, and gender, and detectable at picogram-level RNA quantities. Performance of miRNAs in DNA extractions for body fluid identification was assessed and compared to paired RNA extracts. Detection in compromised samples, limit of detection, and species specificity was evaluated according to developmental validation guidelines. Future work will include expansion of population samples and further validation of the markers. Upon completion, this microRNA panel has the potential for rapid and inclusive discrimination of the body fluids encountered in forensic evidence, and capability for rapid implementation.

This project was supported by Award No. 2016-DN-BX-0163, awarded by the NIJ, Office of Justice Programs, U.S. DOJ to SJSW. The opinions, findings, and conclusions or recommendations expressed are those of the authors and do not necessarily reflect those of the DOJ.

Keywords: Biological Samples, Forensics, Genomics, Nucleic Acids
Application Code: Homeland Security/Forensics
Methodology Code: Chemometrics
We are developing particle-based luminescent temperature sensors that can be embedded in building materials such as paint, and be used in case of fire and arson investigations to help determine the temperature and heating duration that occurred during the fire. In addition, these sensors can also be used during testing in fire laboratories for a better understanding of surface temperatures.

The motivation behind our development is twofold. First, as part of a fire/arson analysis, investigators are often trying to determine temperature and burn-time, as this information can provide clues to how and where the fire started and how it evolved. Investigators use indicators such as color and deformation patterns of various materials, including discolored or melted metal, crazed glass, depth of char, spalling, etc. to extract temperature and burn-time information. However, in many cases these indicators are ambiguous at best and false at worst. Second, fire tests under controlled conditions typically use thermocouples to measure and record temperatures. While they work well to measure gas temperature, they have some inherent disadvantages for measuring surface temperatures.

Our sensors consist of lanthanide-doped metal oxide precursors. As these materials are heated, they undergo irreversible transitions such as decomposition, nucleation, grain growth, and phase transitions, changing the crystal field around the lanthanide dopants. Upon excitation with an appropriate wavelength, the sensors emit light that is characteristic of the dopant. However, the specific wavelengths, line widths, intensities, intensity ratios, etc. depend strongly on the condition of the host material. As the sensor material undergoes temperature-induced changes, the emission properties change. Using laboratory-based calibrations, temperature and heating duration can now be determined.
Recent research advances in modifying and controlling cells’ DNA have created a booming field of biological engineering called synthetic biology. In synthetic biology, engineers manipulate and modify living organisms to alter their functionality, sometimes creating entirely novel behaviors. Early successes have shown promise in developing new fuel sources, pollution mitigation, and intelligent drug delivery systems. In synthetic biology, designs are first built using biological modeling and then implemented in a laboratory. These synthetic organisms can be considered living programs that can sense, respond, and interact with humans while they persist in the natural environment. We argue that we should view these as safety-critical devices, which should be regulated and certified in order to assure their safety and restrict the potential for illegal use. Because synthetically engineered organisms follow a cycle of reproduction and replication, they can mutate, and adapt to environmental changes and evolve new behaviors over time. In this poster, we propose using an assurance case, an argument structure often used in other safety-critical systems to reason about safety, and we introduce an orthogonal dimension we call the "Assurance Timeline." The Assurance Timeline can be used to reason about the dynamic, evolving aspects of these systems. We present a case study based on a real application to illustrate our ideas.

**Abstract Text**

**Keywords:** Biotechnology, Quality Control, Sampling

**Application Code:** Safety

**Methodology Code:** Computers, Modeling and Simulation
We describe several chemical methods of hair analysis that can be used to provide characteristic traits about human donors. In one approach, the abundance of amino acids in hair is used to predict sex, age and geographic origin of a hair donor, or to distinguished diabetic patients from a control group. In an alternative method, stable carbon isotope ratios of amino acids in hair is used to classify donors based on body mass index and age group, among other traits.

In both approaches used here, human hair from known donors was first washed and hydrolysed using acid hydrolysis. In one approach, the absolute abundance of the amino acids in human hair was determined by GC-MS of derivatized free amino acids from the hair hydrolysates. In the second approach, bulk and amino-acid-specific carbon isotope ratio analysis was used as input variables for classification. Statistical techniques such as canonical discriminant analysis (CDA) were used to overlook the covariance of amino acid values between individuals caused by dietary factors and instead highlight the selective differences caused by grouping factor(s) such as age, body mass index and sex. Using leave-one-out cross-validation, CDA is able to predict the body mass index of donor’s hair sample with about 80% success rate. Using leave-one-out cross-validation, age group and sex can be predicted with better than 80% success rate.

The compound-specific isotope ration approach has also been applied to the analysis of amino acids in blowflies. We show that blow fly larvae, pupae and adult flies can all be linked to specific meat sources (carrion) because the extent of fractionation for each amino acid is either negligible or reproducible. This latter work demonstrates that blowflies can be used as a proxy for human flesh because the carbon in the flies originates in a predictable way from their human host.

Keywords: Forensics, Forensic Chemistry, Isotope Ratio MS, Mass Spectrometry
Application Code: Homeland Security/Forensics
Methodology Code: Mass Spectrometry
Picric acid (2,4,6-trinitrophenol) is a military explosive used for the manufacture of weapons and fireworks. The detection of this compound is highly relevant for forensic investigations, national security and environmental health application.[sup][1,2][/sup] In this present work, the explosive detection was performed using a new paper-based carbon device fabricated using a CO[sub]2[/sub] laser scribing process, without the need of chemical reagents or controlled environment conditions. The disposable sensor shown good electric conductivity and a design comprising all the three electrodes pattern (counter, working and reference). For the electrochemical characterization, Differential Pulse Voltammetry (DPV) method was optimized in order to achieve the best analytical figures of merit to quantify the picric acid. The best DPV parameters (Step (E[sub]s[/sub]) and Amplitude Potential (E[sub]a[/sub])) were E[sub]s[/sub] = 9.0 mV, E[sub]a[/sub] = 70.0 mV. The interference of some well-known interfering species such nitrate, sulfate and iron were evaluated and non-interference was observed for picric acid detection. The analytical curve under the optimized conditions ranged from 0.48 to 6.9 mmol L[sup]-1[/sup] and limit of detection was estimated as 0.16 mmol L[sup]-1[/sup].

Financial support: FAPESP, CAPES and CNPq.

Keywords: Forensic Chemistry, Laser, Sensors, Voltammetry
Application Code: Homeland Security/Forensics
Methodology Code: Electrochemistry
Analyzing sensitive materials such as latent fingerprints (LFPs) has always been of paramount importance to forensic scientists both for the morphological visualization of the ridge patterns left behind by an impression and for the extraction of chemical information on foreign material. Because fingerprints have long been the gold standard for personal identification in forensic investigations, methods for cultivating and enhancing the visualization of LFPs are continuously evolving. One important challenge is to identify suspicious chemicals present in fingerprint residues, which requires chemical imaging capability.

Recently, vibrational spectroscopy has shown that LFP analysis through tape-lift, Raman mapping, and multivariate data analysis presents a useful tool for forensic investigation. However, there are still major difficulties in terms of acquisition speed, poor spatial resolution, and lack of sensitivity. In this presentation, I will demonstrate the feasibility of using non-destructive, label-free stimulated Raman scattering (SRS) microscopy to quickly and easily extract LFP patterns from different substrates. Contrary to what has been reported, no obvious fingerprint degradation or lipid diffusion is observed with either glass or stainless steel substrate. Importantly, we demonstrate that trace exogenous chemicals can be detected in fingerprints. Furthermore, we present a novel approach of chemically imaging an LFP directly off transparent adhesive tape, bypassing the need for dusting and staining to further simplify visualization and eliminate cross contamination.

Keywords: Detection, Forensics, Raman Spectroscopy, Vibrational Spectroscopy
Application Code: Homeland Security/Forensics
Methodology Code: Vibrational Spectroscopy
Library and museums preserve a wide range of paper materials from their collections. Knowing the composition of the paper, including elemental composition, greatly helps to improve their ability to preserve paper-based objects from the collections. Paper from a collection of books, for instance, may be tested using small (0.1-0.2 g) samples. The results of trace metal analyses can shed light into the production methods used to produce the paper as well as indicate potential problems as some elements are known to induce degradation. A method developed for forensic applications was utilized to examine samples from the William James Barrow book collection, which contains books from the years 1507 to 1899 that have been studied both by Barrow’s lab in the 1960’s as well as the Library of Congress as part of an ongoing research program. Pages were tested with both text and without text to account for any metals within the ink that was used. Samples from 12 different books from different years were digested and analyzed using Inductively Coupled Plasma-Optical Emission Spectroscopy and then the data was compared for any differences across the examined materials.
Abstract Text
The fire debris analysis offers vital evidence to a forensic investigation. It helps to validate any suspicion of the intentional use of ignitable liquids to initiate a fire or accelerate it. Analysis of materials from crime scenes requires reliable sample preparation in order to efficiently determine the presence of ignitable liquid residues (ILRs). Several well-established methodologies have been approved by the American Society for Testing and Materials (ASTM). Although recent various improvements, some of these methods still have associated drawbacks.

In this study, we present results of the application of Micro-Chamber/Thermal Extractor (µCTE) to the analysis of ignitable liquids (diesel and gasoline) in fire debris. We tested ignitable liquids on two substrates: Wooden dowel and Tea towel cloth. We determined that the µCTE not only has the advantage of rapid sampling but allows to have a great level of control over the sampling conditions for more consistent profiles. Moreover, it offers high sensitivity advantages associated with pre-concentration thermal desorption (TD) and gives the analyst the ability to easily modify the method and develop ideal sampling conditions depending on the suspected accelerant or the material being analysed.

Ensuring legally defensible evidence in arson investigation, sample re-collection option allows to achieve multiple re-run of the same sample. Furthermore, it conserves both the fire debris sample and the TD tube. We also assessed the carryover effect for the diesel and gasoline samples prior to the next sample analysis and the results were less than 0.050%, which could be considered difficult for other systems.

Keywords: Forensics, GC-MS, Sample Introduction, Thermal Desorption
Application Code: Homeland Security/Forensics
Methodology Code: Gas Chromatography
Abstract Text
This presentation will overview progressive data from NIJ-funded research using culture-based methods, high-throughput molecular analyses, and innovative collaborations between scientists and forensic practitioners investigating changes in human post-mortem microbiome (HPMM) structure and function, to correlate decomposition of a once living host, and estimated PMI.

Part one of this presentation describes HPMM community, transmigration, and gene expression of commensal bacterial species in a controlled setting using quantitative PCR, and next generation sequencing. Results suggest bacterial transmigration and expression, and specific microbiome profiles may be sufficiently predictable for estimating a PMI range.

Part two focuses on describing data and demographics of a HPMM Database. In a first-of-its-kind effort, we have collected swab samples from over 1,000 bodies received during routine death investigations from partnership with a major, metropolitan city medical examiner’s office in Michigan. Metadata and demographics to be discussed for the database are as follows: sex, ethnicity, age, location at the time of discovery, date and time pronounced dead, the autopsy date, stature of a body, the manner and cause of death, and the estimated PMI range. Also, microbiomes of a subset of 120 cases show important associations of specific microbial communities and individual taxa with PMI estimates and manner of death. Results suggest that the HPMM is highly variable when evaluated within the context of routine case investigations of a large city population, but that HPMM profiles emerge that are potentially useful for forensic science.

Altogether, this project greatly expands on early HPMM studies, and demonstrates potential real-world utility of microbes in forensic science. Samples from this largest known human postmortem microbiome dataset to-date will serve as the foundation for further studies and research into the microbiome after death.

Keywords: Biological Samples, Forensics
Application Code: Genomics, Proteomics and Other 'Omics
Methodology Code: Sampling and Sample Preparation
### Development of a Paper Analytical Device for the Detection of Illicit Drugs

The presumptive drug tests currently used by law enforcement officers in the field are often critiqued by the public due to their high false positive rates and subjective interpretation. Each test pouch uses a colorimetric chemical reaction to identify the illicit drug, however, many other substances such as cutting agents can interfere with the reagents. To combat this, a paper-based analytical device (PAD) has been developed and combines 12 colorimetric tests to create a “color-barcode” for each illicit substance. The PAD uses less solid than current presumptive tests, costs less than the test pouches, and can be used in fewer than 5 minutes. A portable light box ensures consistent lighting on the PAD so the colors can be read accurately regardless of the environment. A limit of detection study for cocaine, crack cocaine, heroin, and methamphetamine and a blinded sample study will be discussed.

**Keywords:** Drugs, Forensic Chemistry, Lab-on-a-Chip/Microfluidics

**Application Code:** Homeland Security/Forensics

**Methodology Code:** Microfluidics/Lab-on-a-Chip
For decades, law enforcement officers have been using color tests to presumptively identify suspected drugs. In recent years, color tests have received negative media coverage for false positives resulting from testing donut glaze, dry wall, sugar, tea leaves, soap, and other non-illicit substances. Handheld Raman devices have shown to be a superior testing method, but the price for one device is not affordable for most law enforcement agencies. This work stems from prior NIJ-funded research and seeks to further develop a drug-indicating test strip, handheld fluorescence spectrometer to analyze the test strip, and a mobile app with access to a reference database of drug standards. The drug-indicating test strip is made using copper(I) iodide, a compound known to form fluorescent products with amines (specifically alkaloids). The copper(I) iodide testing method yields unique photoemission data for each substance based on its chemical structure, allowing for the discrimination between similarly-structured controlled substances and designer drugs. With the help of data matching software, this method will hopefully provide law enforcement officers with a better method for presumptive field drug testing and crime lab analysts a method for high-throughput drug screening.
The Swept-Wavelength Optical Raman Device (SWOrRD) is an NRL patented spectroscopy platform that combines the advantage of resonance-Raman spectroscopy with multi-wavelength operation. This instrument rapidly illuminates samples at multiple laser wavelengths from deep-UV to the near-infrared and is used to generate two-dimensional Raman signature maps of materials.

We have obtained Raman signature maps of controlled substances and cutting agents using deep-UV illumination wavelengths. Illuminating at multiple wavelengths not only provides information about the resonance behavior of target substances but also can potentially provide better identification of these substances in mixtures. We will discuss sample characterization, sample degradation considerations and limits of detection.

The goal of this research is to use SWOrRD as an analytical technique for the rapid identification of controlled substances in mixtures.

Keywords: Data Analysis, Forensics, Quantitative, Raman Spectroscopy
Application Code: Homeland Security/Forensics
Methodology Code: Molecular Spectroscopy
Non-Invasive Identification of Body Fluid Samples Using Infrared Spectroscopy and Innovative Chemometric Strategies for Forensic Applicability

Vibrational spectroscopy has shown great potential to identify body fluid (BF) samples non-destructively, based on the characteristic spectral patterns. In order to improve the applicability to more practical forensic demands, we explored multivariate spectral analysis for objectively discriminating BFs, involving soft response against unexpected samples and robust response against sample-aging.

ATR FT-IR spectra from five types of BFs, blood, saliva, semen, urine and sweat, showed the characteristic spectral patterns with donor- and spatial-variations. A multivariate discriminant modeling method, Partial Least Squares-Discriminant Analysis (PLSDA), enabled to classify the spectral patterns of five body fluid types correctly. In addition, the combination with Q-statistic technique after PLSDA spectral regression allowed the soft response to exclude unexpected sample’s spectra as outliers.

Aging for several months caused distortion of the spectral patterns of BFs. The standard multi-class discriminant model constructed with fresh BFs’ data was not effective to discriminate the aged BFs due to lack of learning of aged BFs’ spectra and low efficiency of modeling. Then, we proposed a novel model structure using dichotomous classification tree, where a two-class discriminant model was equipped at each node. The proposed model built with fresh BFs’ data drastically improved the discrimination accuracy for aged BFs. Moreover, the novel model was beneficial to reduce the experimental cost for model construction against sample-aging.

The combination of vibrational spectroscopy and chemometric strategies aiming for forensic requirements provides an innovative approach to identify BFs non-invasively with advantages of objectivity and soft and robust applicability.

This work was supported by the Japan Society for the Promotion of Science (Grants-in-Aid for Young Scientist (B) 17K18380 to A. T. and Grants-in-Aid for Scientific Research (S) 26220805 to T.O.).

Keywords: Biological Samples, Chemometrics, Forensics, FTIR
Application Code: Homeland Security/Forensics
Methodology Code: Chemometrics
The STR Sequencing Project (STRSeq) was initiated to facilitate the description of sequence-based alleles at the Short Tandem Repeat (STR) loci targeted in human identification assays. STRSeq data are maintained as GenBank records at the U.S. National Center for Biotechnology Information (NCBI). Each GenBank record contains: observed sequence of an STR region, annotation of the repeat region (“bracketing” consistent with the guidance of the International Society for Forensic Genetics) and flanking region polymorphisms, information regarding the sequencing assay and data quality, and backward compatible length-based allelic designation. STRSeq GenBank records are organized within a BioProject at NCBI (www.ncbi.nlm.nih.gov/bioproject/380127), which is sub-divided by Commonly used autosomal STR Loci, Alternate autosomal STR Loci, Y-chromosomal STR loci, and X-chromosomal STR loci. Each of these categories is further divided into locus-specific projects. The BioProject will initially contain aggregate alleles across 4,612 samples submitted by four laboratories: National Institute of Standards and Technology (NIST, the project organizer), University of North Texas Health Sciences Center, Kings College London, and University of Santiago de Compostela. In addition to providing a framework for communication among laboratories, the ability to search the BioProject can be leveraged as QC for rare sequences encountered in forensic casework. Future plans for this NIJ-funded effort include a pathway for researchers to submit additional alleles and customized interface tools.

Keywords: Bioinformatics, Forensics
Application Code: Homeland Security/Forensics
Methodology Code: Laboratory Informatics
Natural and man-made mass disasters often result in large numbers of casualties. One of the most important considerations following a mass fatality event is victim identification. However, identification efforts may be complicated by harsh environmental conditions, limited facilities, loss of electricity and refrigeration. If remains cannot be stored or identified quickly, the body decomposes and the DNA in those tissues degrades making DNA typing more difficult. This project investigated the effectiveness of various in-field methods for collecting DNA from decomposing human remains.

In addition, several alternate DNA preservation, purification, and amplification strategies were also tested in order to facilitate faster, more direct DNA identification. Skin and tissue samples were collected from three decomposing human cadavers over a two-week period at the Southeast Texas Applied Forensic Science Facility in Huntsville, Texas. Three protocols were used to collect DNA from decomposing cadavers in the field: 1) swabbing skin with 4N6FLOQSwabs, cotton, and foam swabs, 2) inserting a swab into a small incision in the thigh, and 3) skin/muscle biopsy. Biopsy punches were compressed onto FTA Elute cards prior to storage or stored in a liquid tissue preservative that facilitates leaching of DNA into solution for quicker and more direct amplification. All samples were stored at room temperature for one month. Samples were processed in two ways: 1) standard forensics DNA workflow including DNA extraction, quantification, amplification, and capillary electrophoresis and 2) rapid purification and/or direct amplification. DNA quantification and STR typing data will be presented in order to compare the success of each sampling, storage, and processing strategy. The novel combination of in-field sample collection methods, tissue preservation and more rapid, direct sample processing has great potential for forensic application and ultimately criminal justice investigations.

Keywords: Biological Samples, Capillary Electrophoresis, Forensics, Identification
Application Code: Other
Methodology Code: Capillary Electrophoresis
Routine screening techniques are employed by crime labs for identifying controlled substances in seized drug samples and determining the samples that need to be further analyzed using confirmatory techniques. These screening techniques commonly include quick colorimetric tests and UV or fluorescence spectrometry, all of which falls under Category C in SWGDRUG’s categorization scheme. Although these techniques are rapid, they have the lowest potential discriminating power. In order to increase laboratory efficiency and turnaround time, screening techniques with higher potential discriminating power is desirable. Use of a Direct Analysis in Real Time - Mass Spectrometry (DART-MS) system for more accurate screening for controlled substances with speeds comparable to techniques such as color tests and FTIR is presented.

In order to utilize less accurate mass data for these measurements we have developed a DART-MS database that is up-to-date with the most current drugs. All data were acquired using a more cost-effective platform for use in forensic laboratories. We demonstrate here a program that enables the creation and search of the new low resolution DART-MS databases. A continual effort is being made to develop the database to include a wide array of controlled substances and drugs of abuse using certified reference materials for approximately 250 substances, which includes primarily opioids, cannabinoids, stimulants, benzodiazepines, novel psychoactive compounds. Preliminary tests thus far have shown no false negatives and all false positives were attributed to isomeric compounds. Potential for use of this system in the mobile laboratory setting using a thermal desorber and real time analysis software will be discussed.

Keywords: Drugs, Forensics, Forensic Chemistry, Mass Spectrometry
Application Code: Homeland Security/Forensics
Methodology Code: Mass Spectrometry
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<td>Primary Author</td>
<td>Jose R. Almirall</td>
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Abstract Text
The Center for Advanced Research in Forensic Science (CARFS) is an NSF/NIJ-funded multi-university Industry University Cooperative Research Center (IUCRC) program that enables industry-relevant, pre-competitive research via a multi-member and sustained partnerships among industry, academe, and government. FIU’s CARFS Center includes faculty and students from five different academic institutions (FIU, Northeastern, George Washington, Texas A&M, and University of South Alabama) with more than 100 faculty and students participating. The Center also includes 21 active industry board members representing large, medium and small companies as well as State/Federal/Local government and non-profits. The CARFS mission is to support and perform cutting-edge, pre-competitive fundamental research in science, engineering, and technology areas of interest to industry that will drive innovation and the U.S. economy. The IUCRCs offer a platform for significant leveraging of financial investment by members to accelerate the forensic science knowledge base in emerging technologies and manufacturing sectors and develop an industrially savvy workforce to benefit the forensic science enterprise and the US economy. The CARFS have funded more than 25 faculty with ~ $ 500.k of seed funding to initiate 19 research projects of interest to the industry advisory board. To read more about CARFS, please go to: www.forensic-research.org

A summary of the CARFS, IUCRC and a list of the funded research projects will be described.

Keywords: Forensics, Forensic Chemistry
Application Code: Homeland Security/Forensics
Methodology Code: Education/Teaching
NIJ Forensic Science Research & Development Poster Session

Rapid Direct PCR: A Method for Complete Sample Processing Obtaining STR Based Genotypes in 15 Minutes or Less

It is often extremely important to rapidly screen suspect samples at border controls and police stations where the individuals in question can only be detained for short periods of time. Current DNA typing methods provide the best biometric information yielding identity, kinship and geographical origin, but they are not sufficiently fast to permit identity of a suspect DNA in real time. Current rapid DNA systems take about 90 minutes and involve complex extraction and analysis procedures.

This time for sample processing can be greatly diminished with the use of rapid and direct PCR methods which make use of new mutant polymerases, designed for increased processivity as well as resistance to inhibitors, and new thermal cycler designs can make the analysis even faster. With the combination of these enzymes and instruments we have created a method for multiplexed PCR amplification in under 10 minutes. When coupled with microfluidic electrophoresis, we can produce complete genotypes in 15 minutes.

To do this we have been testing specially engineered enzymes Z-taq and Omnitaq along with direct buffer systems to rapidly amplify a 7 loci STR multiplex with no extraction step needed. The designed multiplex includes D5S181, D13S317, D7S820, CSF1PO, D16S539, Penta D and Amel which have sizes between 106 and 454 bp in size. By using off the shelf components and commercially available enzymes it is possible to create a procedure that acts as a quick, highly informative sample screening process that also retains sufficient DNA for later manual processing using standard STR.

The results of this study demonstrates the application of ultrahigh speed direct PCR for the successful amplification a 7 loci multiplex. With such a procedure in place any crime lab can produce a nearly instantaneous genotype from buccal samples. Additionally instruments are easily portable and so genotypes could also be obtained from onsite locations such as airports of sites of mass disasters.

Abstract Text

Primary Author
Georgiana Gibson-Daw
Florida International University

Co-Author(s)
Bruce McCord

Keywords: Bioanalytical, Capillary Electrophoresis
Application Code: Bioanalytical
Methodology Code: Capillary Electrophoresis
Novel Aspect: A portable acetone-assisted photoionization and chemical ionization ion trap mass spectrometer was designed and characterized for on-situ analysis of illegal drugs.

Introduction: The portability, sensitivity and accuracy are tough issues for mass spectrometry to achieve on-situ analysis. Ion trap coupled with discontinuous atmospheric pressure interface realized the portability. A novel ionization source based on acetone-assisted photoionization and chemical ionization was designed and implemented to realize the high sensitivity and accuracy for on-site rapid detection of illegal drugs.

Methods: A commercial 10.6 eV VUV krypton (Kr) discharge lamp with acetone dopant gas was used for soft positive ionization. Liquid and solid sample was collected on a thin film of PTFE, and dry air carry the gaseous samples generated from thermal desorption sampler into the ionization chamber. Acetone is added into the ion source and assists the ionization of the analytes.

Results: Twenty-seven different illegal drugs were tested, the ionization source is soft and their characteristic ions are [M+H]+. The analysis time is less than 2 min, and the LODs of the drugs are at pg level for standard samples. The addition of acetone improve the signal intensity as high as 18-fold for methamphetamine. The accuracy of the drug identification is improved by using tandem mass spectrometry analysis, and the pattern recognition of drugs was carried out using precursor ion and characteristic fragments. The qualitative accuracy of illegal drug components in plant-based drug samples (such as opium ointment) was improved. Illicit drugs samples in drink were also detected.

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Keywords: Chemical Ionization MS, Ion Trap, Tandem Mass Spec, Thermal Desorption
Electronic cigarettes were developed as a method for nicotine delivery. When the e-cigarette is activated, the e-liquid is aerosolized and inhaled. Cannabidiol is a significant active ingredient of *C. sativa* and *C. indica* and has been purported to have anti-convulsant, anti-nociceptive, and anti-psychotic properties. CBD has not been approved by the FDA for medical purposes. 5-fluoro MDMB-PINACA (5F-ADB) is reported to have high cannabimimetic activity. In 2014, the Drug Enforcement Agency (DEA) reported 2,311 incidents involving medical intervention or death from 5F-ADB, and, in January 2017, placed it on Schedule I.

Two commercial e-liquids were submitted to our laboratory for analysis. The same two products and an additional product were then purchased directly from the manufacture. These e-liquids were analyzed using a Direct Analysis in Real Time Mass Spectrometry (DART-MS) and a Gas Chromatography Mass Spectrometry (GC/MS). Active ingredients were identified using commercial standards based upon retention time and mass spectral comparisons.

All the e-liquids were determined to contain CBD. Three of the four were determined to contain 5-fluoro MDMB-PINACA and the other was determined to contain dextromethorphan, a cough suppressant. These e-liquids were advertised to contain only CBD. The website where they were purchased from stated that these products do not use synthetics or other psychoactive compounds in their products. 5F-ADB can lead to dangerous consequences, particularly where the users are unaware and are using for therapeutic reasons.

This project was supported by NIJ Award No. 2016-DN-BX-0150, awarded by the National Institute of Justice, Office of Justice Programs, U.S. Department of Justice and the National Institutes of Health Award No P30DA033934. The opinions, findings, and conclusions or recommendations expressed in this abstract are those of the author(s) and do not necessarily reflect those of the Department of Justice.

Keywords: Consumer Products, Drugs, Forensic Chemistry, Mass Spectrometry
Application Code: Homeland Security/Forensics
Methodology Code: Mass Spectrometry
As the recreational use of marijuana becomes more common in response to legalization, there is a need to address the safety issues associated with cannabinoid impairment behind the wheel and at the worksite. Law enforcement officers and others must have tools to quickly and conveniently assess recent marijuana use and link it to impairment, much like alcohol breathalyzers that allow rapid indication of alcohol impairment. Breath capture of tetrahydrocannabinol (THC) is a new method for testing during the presumed impairment window that offers significant advantages over oral fluid or blood sampling in roadside and workplace environments. We have developed a novel portable instrument that uses fluorescence detection to quantify the amount of THC in breath. We have tested the performance of this system in human studies under institutional review board approval.

We will describe the design and operation of the automated instrument and the associated chemistry assay. The system captures breath samples on a substrate, elutes the substrate with a solvent, binds THC with a fluorescent label, and isolates the bound THC for quantifiable optical detection. The device is suitable for use in roadside tests and in ongoing lab studies.

Our human studies have allowed us to elucidate factors that affect the amount of THC available for capture from breath. Kinetic data will be presented showing the range of THC levels under various conditions. We demonstrate that the level of detection in breath needed to determine marijuana use throughout the window of impairment is below 50 picograms per liter of breath. Next, we will show results that validate our fluorescence chemistry against gold-standard mass-spectrometry measurements.

We have begun testing to establish the correlation between breath levels of THC and impairment. We will discuss the field use of the device in multiple high-speed track tests where we show for the first time breath levels of THC in impaired drivers.

**Keywords:** Detection, Drugs, Environmental/Biological Samples, Fluorescence

**Application Code:** Homeland Security/Forensics

**Methodology Code:** Portable Instruments
Ensuring the performance and safety of body armors during service is crucial for institutions such as the United States National Institute of Justice (NIJ). The NIJ’s commitment to safety prompts the research of the degradation of high performance fibers like Kevlar, Twaron and Dyneema. Degradation, the reduction of the mechanical properties such as strength and toughness, occurs when a material is exposed to various common environmental factors like sweat, humidity, temperature and sunlight. In order to understand how one of these factors affects fibers, it is isolated from other. Currently, the effects of water and pH are studied. The amount of water and its effects on the fibers mechanical properties are tested through tensile testing and dynamic mechanical analysis (DMA). Single fibers and yarns are tested through these destructive methods, however, a non-invasive method that uses anti-particles can be used to detect changes in the structure of fibers as they deteriorate. Positron annihilation lifetime spectroscopy (PALS), emits positrons (the antiparticle of an electron) which reside in low electron density areas such as the free volume of polymers and voids. Once a positron interacts with an electron, they bound and form a new particle called the positronium. The time between the emission of a positron and the detection of positronium particle is related to the size of the low-density area. As the fibers degrade, areas such as the free volume also change in dimensions. A distribution of the size and amount of free volume is offered through PALS, which can then be used as a complementary technique to the previously mention mechanical tests. Comparing the changes in free volume to those in the mechanical properties gives a broader understanding of the degradation process and rate for high performance fibers, and can later be implemented for the lifetime prediction of new and used body armors.

Keywords: Characterization, Materials Characterization, Material Science, Polymers & Plastics
Application Code: Material Science
Methodology Code: New Method
Ion mobility spectrometers (IMS) are systems which can detect many different toxic industrial chemicals in very low concentrations. Their ambient pressure operation makes them suitable for small, portable hand held systems. Other important parameters are selectivity and cost when talking about application out of the lab.

In this talk we will address the selectivity of small IMS devices. Two different approaches based on ion mobility are known for separation of ions in an electric field. The classical time of flight measurement at low electric field strength (TOF-IMS) and filtering of ions at asymmetric low and high field strength (FAIMS or DMS). Both methods use different properties of the ions for separation. We investigated the possibility to combine the two methods in one drift tube. We will present theoretical considerations and numerical simulations of the approach. As well we will present the first measurements with an IMS that utilizes the separation using the TOF approach superimposed by the FAIMS separation. We will demonstrate the ability of the new approach to separate ions, which are not distinguishable in a classical TOF-IMS because of the same low field mobility (i.e. same K0). This approach enables one to combine orthogonal ion separation mechanisms in one drift tube.

In the second part of the talk we will address the costs of building an IMS tube. We will introduce a low cost printed circuit board (PCB) IMS. Using this state of the art production technology makes it possible to achieve very low material costs, while maintaining analytical purity and good performance. Based on a low cost IMS we will introduce a new hand held detection system – GDA-P. To extend the detection capabilities of the system the GDA-P includes selectable other orthogonal sensor such as a dedicated electrochemical cell (EC) or alternatively a photo ionization detector (PID).

**Keywords:** Detection, Instrumentation, Integrated Sensor Systems, Sensors

**Application Code:** Homeland Security/Forensics

**Methodology Code:** Integrated Sensor Systems
We describe the development, optimization, and application of a novel hyphenated technique, Sequential Injection Analysis-Nuclear Magnetic Resonance Spectroscopy (SIA-NMR). SIA-NMR combines the automated sample pretreatment capabilities of SIA with the qualitative power of proton NMR to create a high throughput, small footprint and low cost instrument with applications in a variety of areas, including forensic science. Over the past decade, the emergence of “New Psychoactive Substances” (NPS) such as synthetic cathinones have created a confounding problem in forensic analysis. Small structural modifications to traditional psychoactive substances circumvent laws and foil presumptive testing techniques, such as color tests, thin layer chromatography, and infrared spectroscopy. Historically, NMR has been underused in the forensic laboratory primarily owing to its poor sensitivity and selectivity as well as its high cost and large footprint. Recently, low-field benchtop proton NMR spectrometers have emerged; however, their small footprint is also accompanied by relatively poor sensitivity and selectivity. In addition to interfacing SIA to proton NMR, we are studying the use of solid-phase extraction (SPE) and liquid-liquid extraction (LLE) in the SIA manifold to pre-concentrate samples and remove potential matrix interferences. We found strong cation-exchangers to be optimal SPE sorbents for amphetamines such as the cathinones, and we will describe the optimization of the SIA-SPE method in terms of loading and elution conditions. We will also describe our results for on-line LLE, particularly in terms of phase selection, extraction conditions, and system timing. NMR spectra of NPS simulants were also obtained to determine the analytically useful peaks in terms of intensity, selectivity, and linearity. This work was sponsored by the Research Growth Initiative at UW-Milwaukee.