The Wallace H. Coulter Lecture

Integrated Brainwide Structural and Functional Analysis

Deisseroth will discuss the development of optogenetics (a technology for controlling millisecond-scale activity patterns in specific cell types using microbial opsins genes and fiberoptic-based neural interfaces, all in freely-behaving animals including adult mammals). He will also speak on the development of hydrogel-tissue composites (e.g. his CLARITY method for creating composites of biological molecules in tissue covalently linked to acrylamide-based hydrogels, allowing removal of unlinked tissue elements to create transparency and accessibility to macromolecular labels; the resulting new structure allows high-resolution optical access to structural and molecular detail within intact tissues without disassembly). He will also discuss the application of his methods to discover the neural cell types and connections that cause adaptive and maladaptive behaviors.

Keywords: Bioanalytical, Biological Samples
Application Code: Bioanalytical
Methodology Code: Education/Teaching
Dissolved organic matter (DOM) is a complex amalgam of heterogeneous molecules, such as humic and fulvic acids, polysaccharides, proteins, lipids, nucleic acids, soluble microbial products and synthetic organic chemicals of urban, agricultural and industrial sources, and it could be considered as a sample fingerprint. It characterization and monitoring of changes along the water treatment process, or in case of environmental waters monitoring of temporal and spatial changes of its profile enables gaining deeper understanding of wastewater treatment or of processes occurring in the natural environment.

Herein we describe the development and application of a non-targeted analysis of DOM in environmental and wastewater using liquid chromatography-high resolution mass spectrometry (LC-HRMS) data. A streamlined workflow, for the processing of LC-HRMS data and extraction of signals, prediction of elemental formulas and signal alignment, is designed based on commercial software tools and a statistical programming tool resulting in a range of variables calculated for the obtained substances such as: (i) atomic ratios X/C with X=H, O, N, etc. and van Krevelen plots (atomic ratios H/C vs O/C) which grouped the constituents into clusters depending on their assigned elemental composition, (ii) degree of unsaturation (DU, DU/C), (iii) amount of %CHO, %CHON formulas in a sample, (iv) Kendrick mass, Kendrick mass defects (KMD) to uncover homolog series for multiple addition/elimination moieties and trace the differences, and (v) statistical variables (e.g. principal components that uncover similarities between samples), among others. The use of this approach has proven useful in characterizing organic matter composition through a synergistic, fast approach to data treatment on the level of bulk DOM, its fractions and individual constituents that would otherwise involve multiple analytical techniques.

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**Keywords:** Liquid Chromatography/Mass Spectroscopy, Monitoring, Statistical Data Analysis, Water

**Application Code:** Environmental

**Methodology Code:** Liquid Chromatography/Mass Spectrometry
With rapid growth in population and expansion of commerce, our wastewaters contain thousands of compounds. Hence, non-targeted analysis using High Resolution Accurate Mass (HRAM) instrument is gaining traction. In addition to monitoring thousands of analytes, HRAM instruments collect data on all ionizable species thus allowing retrospective data-mining to determine new and yet unknown contaminants that may become relevant.

Current approach employed 1290 UHPLC and 6545 LC/QTOF with 40uL injection without sample enrichment. All Ions MSMS acquisition and a Water Screening Personal Compound Database and Library (PCDL) that contained >1400 environmentally-relevant compounds were used. The PCDL contained >1,000 compounds with MS/MS spectra that included EPA regulated compounds, pharmaceuticals, pesticides, veterinary drugs, illicit drugs, toxins etc. All Ions MSMS in positive and negative modes was performed at CEs 0, 20 and 40 eV allowing simultaneous collection of precursor and fragment data.

Water samples from a local treatment plant were analyzed: influent, primary effluent after coagulation, secondary effluent after sludge treatment and final effluent after NaOCl treatment. About 50 compounds were identified in influent using Water Screening PCDL. After coagulation, similar number of compounds was identified (~65% overlap) suggesting little removal of contaminants. Activated sludge treatment showed ~40% reduction of compounds, mostly for acidic organics, e.g. ibuprofen. Disinfection with NaOCl resulted in transformation products e.g. dichloroacetic acid, uracil, and guanylurea. Perfluorinated acids, phthalates, metformin, valsartan, gemfibrozil etc. survived treatments and were present in final effluent.

LC-QTOF and Water Screening PCDL is of great potential for comprehensive water quality monitoring during treatment processes. The All Ions MSMS acquisition allows confident analytes identification by fragments and retrospective data mining for new entities.

Keywords: Contamination, Environmental/Water, Liquid Chromatography/Mass Spectroscopy, Mass Spectrometry
Nonylphenol (NP) is a mixture of isomers that is being widely used as detergents, lubricating oils, etc. Wastewater treatment plants are only partially effective in treating nonylphenol and concentrations at the ppb levels are not uncommon. While it is known that nonylphenol is amendable to oxidation by chlorine and monochloramine, further study on the formation and pathway of disinfection by-products in the presence of bromide and iodide is needed.

NP in a buffered system containing bromide and iodide was oxidized with three commonly used disinfectants; chlorine, monochloramine, and chlorine dioxide. Exposure times were between 10 minutes to 5 hours. Samples were extracted using LLE w/hexane for the GC analysis and by SPE for the GC and bioassay analysis. GC-ICP-MS analysis was utilized to confirm the presence of brominated and iodinated species and through RT locking the products were identified using GC-QTOF.

Mono- and dihalogenated DBPs were identified as first by-products. While all combinations of mono- and dilhalogenated DBPs were identified after chlorination, the oxidation of NP with monochloramine and chlorine dioxide was more selective. Mainly chlorinated and iodinated by-products were identified after oxidation with chlorine dioxide, but no brominated ones. Contrary, after chloramination, only iodinated and brominated DBPs were identified. To further evaluate the genotoxicity of the sum of DBPs in each sample, p53-bioassays were analyzed for each of the extracts.

Finally, wastewater effluents from different wastewater treatment plants around the US were analysed to confirm the presence of some halogenated NP by-products.

Keywords: Chromatography, Environmental Analysis, Gas Chromatography/Mass Spectrometry, Liquid Chromatography, Environmental Mass Spectrometry
This presentation will review the most innovative strategies using liquid chromatography time-of-flight mass spectrometry techniques applied to environmental contaminant identification. A brief history on how we got started with accurate mass techniques and how this developed into more routine applications in the environmental field will be presented. Several examples will include analysis of pesticides, pharmaceuticals and their degradation products in several types of waters (surface, groundwaters and wastewaters) and food. Special emphasis will be devoted to the description of specific tools using accurate mass, which have been successfully used to identify new and emerging contaminants in water samples. Some of these tools include the following: the use of molecular feature extraction based software, accurate mass databases, isotope filters, mass defect, use of accurate MS-MS fragmentation and mass profiling. These techniques were successful for finding non-targets and unknowns that had not been previously included in routine target methods. In this presentation, examples on how to maximize the use of these tools will be given for specific new findings in the environment. In addition, high resolution accurate mass using TOF has also been shown to discriminate between several isobaric/isomeric pairs of compounds (same nominal mass/same exact mass), frequently present in water samples. Finally, recent developments in the identification of additives used in hydraulic fracturing waters using accurate mass spectrometry tools will be presented.

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

**Application Code:** Environmental

**Methodology Code:** Liquid Chromatography/Mass Spectrometry
**Abstract Text**

Colloidal inorganic nanoparticles are of great interest for many applications, including biomedical ones (sensors, drug delivery vehicles, therapeutics) and engineering ones (electronics, optics, energy storage). As these materials become more ubiquitous in the environment, it becomes imperative to design them from the outset with sustainable best practices in mind. For entry into the living food web, one fundamental interaction point is the nanoparticle-cell membrane interface. Understanding this interface deeply at the molecular level requires well-defined nanoparticles, well-defined membranes, and multiple analytical and computational tools to measure and predict events. Gold nanoparticles and lipid membranes are chosen as model systems due to their robustness and vast existing literature. Analytical techniques to measure the interaction of gold nanoparticles with various lipids include NMR, nonlinear optical spectroscopy, quartz crystal microbalance with dissipation, super-resolution optical imaging, and electron microscopy. One major finding is that nanoparticles can strip lipids from membranes to form lipid coronas, analogous to the more well-known protein coronas on colloidal nanoparticles. Implications of these results for the future of sustainable nanotechnology will be discussed.

**Keywords:** Adsorption, Lipids, Microscopy, Nanotechnology

**Application Code:** Nanotechnology

**Methodology Code:** Surface Analysis/Imaging
Engineered nanoparticles are increasingly being incorporated into devices and products across a variety of commercial sectors – this means that engineered nanoscale materials will either intentionally or unintentionally be released into the ecosystem. The long-term goal of the presented work is to understand the molecular design rules that control nanoparticle toxicity using aspects of materials science (nanoparticle design, fabrication, and modification), analytical chemistry (developing new assays to monitor nanotoxicity), and ecology (monitoring how nanoparticles enter and accumulate in the food web through bacteria and how these nanoparticles influence bacterial function). Taken together, these data suggest that careful consideration of engineered nanoparticle surface chemistry will likely allow design of safe and sustainable nanoscale materials.

**Keywords:** Bioanalytical, Consumer Products, Environmental, Nanotechnology

**Application Code:** Nanotechnology

**Methodology Code:** Fluorescence/Luminescence
Abstract Text
Understanding the interactions of nanoparticles with biological tissues is central to the development of safe nanotechnology for medical utility inside the body. A key limitation is the lack of available imaging platforms to probe distribution and location of nanoparticles within tissues. This presentation will discuss current advances in high-throughput clearing techniques to render tissues optically transparent to allow nanoparticles to be imaged. We further describe new algorithms and mathematic techniques to enable mapping and quantification of the nanoparticles within tissues that are normally associated with toxicity (e.g., liver, kidney). Availability of these newly developed techniques have enabled us to study how particle design (size, shape, and surface chemistry) determine their in vivo transport, fate and toxicity. These fundamental studies will provide guiding principles to the engineering of nanotechnology and engineered materials for diagnosing and treating cancer and other diseases.

Keywords: Imaging, Material Science
Application Code: Biomedical
Methodology Code: Microscopy
The protein corona on the engineered nanomaterials (ENMs) entering biosystems closely impact their biodistribution and their potential adverse effects. While the corona composition is strongly dependent on the synthetic identities of ENM; the structure, pI, and Mw of the protein as well as binding pockets on surface also play important roles to ENM-protein interaction. Besides, the protein corona may be different as ENM encounter various biological fluids; and interaction between ENMs and cells could change the concentration of molecules in the extracellular fluid, in turn causing ENM aggregation and alter cellular uptake. Such a complex relationship among corona, biological fluid, and ENMs imposes great difficulties on mechanistic studies of the roles of corona in regulating biological responses to ENMs. To overcome this difficulty, my group has developed several techniques to facilitate the study of protein corona. They include a capillary electrophoresis method for measuring the dissociation constants for the protein-ENM complexes; a screening method that takes advantage of a fluorogenic dye to label the primary amines on protein surface; and a flow field flow fractionation (FlFFF) method to remove the unbound/soft corona and obtain the ENMs coated with the hard corona. Using these techniques, we have found out that the type of surface ligand, the diameter of the ENMs, and their composition can strongly affect the affinity and kinetics of protein binding. Certain hard corona proteins could reduce the cytotoxicity of the ENMs; or improve cellular uptake of the ENMs. Overall, we believe these techniques will be useful in identifying the correlation between corona composition and ENM’s physicochemical properties, as well as its biological functions.

Keywords: Bioanalytical, Fluorescence, Isolation/Purification, Nanotechnology
Application Code: Bioanalytical
Methodology Code: Fluorescence/Luminescence
Nanomaterials are integral to innovate commercial products with the annual US production of five engineered nanomaterials estimated to range from 8,000 to 40,000 tons per year. The health effects of nanoparticles remain uncertain due to several challenges. New analytical technologies based on capillary electrophoresis are described that overcome many barriers to nanoparticle analyses. These barriers include complex mechanisms of toxicity related to size, shape, surface characteristics, and composition. These problems are further confounded by evidence that nanoparticle preparations are rarely homogenous and can change, for example through agglomeration, surface adsorption, or dissolution. The physical, chemical, and biological properties of nanoparticles must therefore be characterized prior to evaluating in vitro and in vivo toxicity. The impact of molecular and physiological processes on nanoparticle characteristics must be assessed to shed light on the significance of nanoparticle delivery, mechanisms of uptake, and the potential for modification. Advanced strategies for capillary electrophoresis affinity screening are described to demonstrate novel means of detection, methods that obviate preparation inhomogeneity, and rapid screening of individual preparations to quantify changes in nanomaterial properties.

Keywords: Bioanalytical, Capillary Electrophoresis, Nanotechnology

Application Code: Nanotechnology

Methodology Code: Capillary Electrophoresis
Advances in All-Optical Laser Plasma Spectroscopy

Exploring Mars with Curiosity: New LIBS Applications Out of This World

ChemCam has collected nearly 400,000 LIBS spectra on Mars since the Curiosity rover landed in Gale crater in 2012. Key rover team findings center on Mars’ past habitability, including evidence of a large and long-lasting freshwater lake, and of extensive groundwater interaction in the post-lacustrine era. Curiosity has traveled > 13 km and is steadily climbing the lower portion of Mt. Sharp, a 5 km high sedimentary mound in the center of the 150 km diameter crater. The long-term goal is a sulfate-rich unit representing a later stage in Mars’ geological history. The geological transition to this unit is expected to reveal clues to Mars’ change from a wet and warmer planet to the current cold and dry environment. The rover should reach this unit in the vicinity of a large outflow channel which may also help us understand the origin of Mt. Sharp.

LIBS is uniquely capable of revealing Mars’ geochemistry. Hydrogen observed as a 656 nm emission line allows quantification of the water content of Mars soils and sedimentary rocks. Fluorine found in coarse-grained targets has revealed fluorapatite and mica minerals that washed down from the rim of Gale crater. Along with alkali-, silica-, and aluminum-rich rocks, these have revolutionized our understanding of Mars as an igneous planet lacking plate tectonics but still having low-density continental crust like the Earth. ChemCam’s discovery of boron and evidence for halite (NaCl) provides a picture of the desiccation of Mars lakes as its climate changed. Mn oxides have also been observed. On Earth Mn oxides were not produced until after the rise of atmospheric oxygen. Martian Mn-oxides strongly suggest that Mars also once possessed an oxygen-rich atmosphere.

All 400k Mars LIBS spectra and derived elemental compositions are available at http://pds-geosciences.wustl.edu/missions/msl/chemcam.htm and are described in > 40 peer-reviewed papers.

Keywords: Atomic Emission Spectroscopy, Chemometrics, Geochemistry, Portable Instruments
Application Code: High-Throughput Chemical Analysis
Methodology Code: Atomic Spectroscopy/Elemental Analysis
Laser-induced breakdown spectroscopy (LIBS) is typically performed by focusing a single laser pulse onto the surface of a target. The addition of a second laser pulse to the apparatus adds another dimension to the experiment. Intense laser pulses have been introduced after (and before) the ablation laser pulse to perform dual-pulse LIBS to increase heating and sample atomization in the analytic LIBS plasma. If this secondary laser is deliberately tuned to an atomic transition in an atom or ion in the plasma, this process is called resonance-enhanced LIBS (RELIBS). If the secondary pulse energy is low and the spontaneous emission from the resonantly populated atomic state is observed, this process is called LIBS-LIF (laser-induced fluorescence).

In this talk I will describe our efforts to utilize resonant nanosecond laser pulses from an optical parametric oscillator to investigate these phenomena. In particular, we are interested in the use of LIBS-LIF to preferentially populate specific excited states in singly-ionized neodymium for the purpose of increasing the signal to noise of smaller emission lines that are overlapped by stronger emission lines in the dense LIBS optical spectrum (Figure A). This work is part of a project to make absolute transition probability measurements in lanthanide laser-induced plasmas. A particular level of Nd II at 23229.99 cm−1 with several well-known strong decay branches served as an ideal atomic system for investigating upper state population efficiency as a function of pulse energy, interpulse delay time, and excitation wavelength (Figure B). As well, we are interested in using this system to enhance the ultraviolet emission of singly ionized zinc by resonantly pumping a transition at 589 nm for the sensitive detection of zinc in biomedical specimens and to detect sub-ppm trace concentrations of other elements in bacterial specimens and fish otoliths.

Keywords: Atomic Emission Spectroscopy, Atomic Spectroscopy, Environmental/Biological Samples
Application Code: General Interest
Methodology Code: Atomic Spectroscopy/Elemental Analysis
Advances in All-Optical Laser Plasma Spectroscopy

Progress and Challenges for LIBS in the Deep Ocean and Other High Pressure Environments

Oceanography is rapidly moving from a sampling science, where measurements are made aboard ship on specimens retrieved from the water column or seafloor, to a sensing science, where instrument systems directly make measurements in situ. However, sensor technologies that are required to carry out chemical measurements are not as advanced as in other disciplines. Laser-induced breakdown spectroscopy (LIBS) is an existing in situ technique that can potentially address this shortcoming by extending it to the deep ocean aqueous environment. LIBS uses a pulsed laser and gated detector to measure atomic emission spectra from a laser-induced plasma that forms following laser ablation of the sample.

This talk will include a description of underwater LIBS work at other institutions, but will focus primarily on work being carried out jointly by our group at The University of South Carolina and Woods Hole Oceanographic Institute. The goal of our work is to design a deployable LIBS system suitable for a deep-diving submersible, to measure the elemental composition of deep-ocean hydrothermal vent fluids, measuring ppm levels of alkali and alkaline metal elements at pressures up to 3×10^7 Pa (~2800 m water depth equivalent). Recent studies that will be described include the use of O and H as internal standards in high-pressure water and studies of suspended particle effects. In other applications of LIBS in inaccessible places, planetary applications of LIBS are being investigated at USC including measurements in supercritical CO2 to simulate Venus surface conditions as well as a novel miniature spatial heterodyne LIBS spectrometer for standoff LIBS. This instrument uses a 10 mm diffraction grating for light collection at distances up to 20 meters, corresponding to a collection solid angle of less than one microsteradian.

Keywords: Atomic Emission Spectroscopy, Laser, Plasma, Spectroscopy
Application Code: Other
Methodology Code: Atomic Spectroscopy/Elemental Analysis
Advances in All-Optical Laser Plasma Spectroscopy

The use of nanostructures as ‘spectroscopic enhancers’ is getting a growing interest for several applications in spectroscopy, microscopy and sensing. Many theoretical and experimental works have demonstrated that when a substrate is irradiated with a laser after nanoparticles (NPs) have been deposited on its surface, a local enhancement of the laser electromagnetic field is induced. The recently proposed Nanoparticle Enhanced LIBS (NELIBS) [1-3] is an effective method to improve the sensitivity and decrease the Limit of Detection (LOD) in elemental analysis up to two order of magnitude as compared to traditional LIBS of metal alloys. NELIBS is a variant of the LIBS technique, based on the direct deposition of metal nanoparticles on the sample surface. Signal enhancement in NELIBS, is related to the fact that laser pulse induces coherent oscillation of the conduction electrons in metallic NPs and in turn amplifies the incident electromagnetic field, thereby increasing the electric field near the particle surface. This process allows an enhanced production of seed electrons by electron field emission and consequently a more efficient ablation and plasma excitation. In this work fundamental aspects of NELIBS will be discussed in details, including the sample preparation and the effects of experimental conditions on the NP enhancement of the signal. Finally some example of applications in real world and their perspective will be shown and critically discussed in order to give a complete idea of the potentialities of NELIBS in analytical chemistry.


Keywords: Laser, Nanotechnology, Spectroscopy
Application Code: Nanotechnology
Methodology Code: Sampling and Sample Preparation
### Advances in All-Optical Laser Plasma Spectroscopy

#### Femtosecond Filament-Laser Ablation Molecular Isotopic Spectrometry

Femtosecond filaments have emerged over the past years as an alternative laser ablation sampling source, enabling propagation of pulsed laser beams without diffraction over extended distances. Femtosecond filamentation has been attributed to the dynamic balance between Kerr self-focusing and the defocusing action of free electrons produced by multi-photon ionization of air molecules. Successful demonstrations of the use of filaments for elemental analysis with Laser Induced Breakdown Spectroscopy (LIBS) have been previously reported.

In this talk, we will focus on the detection of isotopes at a distance through the use of Femtosecond Filament-Laser Ablation Molecular Isotopic Spectrometry (F2-LAMIS). The technique combines femtosecond (fs) laser filamentation and ablation based molecular isotopic spectroscopy, allowing isotopic analysis at a distance. Analysis of zirconium (Zr) samples by F2-LAMIS is demonstrated at different distances up to several tens of meters. The molecular and atomic emission intensity, and properties of the filament-induced plasma generated at different filament propagation distances are investigated. Spectral fitting of F2-LAMIS spectra enables semi-quantitative isotopic analysis without the use of calibration standards that is independent of the filament propagation distance for the studied range.

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**Keywords:** Atomic Emission Spectroscopy, Elemental Analysis, Nuclear Analytical Applications

**Application Code:** Nuclear

**Methodology Code:** Molecular Spectroscopy
Advances in Laser Induced Breakdown Spectroscopy

Considerations On Some Uncommon Diagnostics and Analytical Topics in Laser Induced Breakdown Spectroscopy

This talk will address some uncommon topics such as polarization effects of signals and background, the use of photomultiplier tubes in addition to intensified cameras, passive absorption measurements in the early phase of plasma formation, including cavity-enhanced absorption, and the use of excimer lasers (XeCl and ArF) to selectively excite the atomic fluorescence of selected species (e.g., Al and C) in the plasma. These topics have received considerable less attention when compared to other topics like temperature and electron number density measurements, the use of McWhirter criterion to verify local thermodynamic equilibrium, and the increasing use of chemometrics, just to cite a few. Although at first sight the above topics may be considered to be of purely diagnostic relevance, an attempt will be made to demonstrate their potential analytical impact. Examples will include a discussion of the well known Fe spectral interference on the atomic emission of C at 247.856 nm and the use of the PMT to detect the temporal evolution of self-absorption in the plasma using a single laser pulse.

Keywords: Atomic Absorption, Atomic Emission Spectroscopy, Atomic Spectroscopy
Application Code: Other
Methodology Code: Atomic Spectroscopy/Elemental Analysis
The monitoring of industrial processes often calls for techniques capable of measuring the composition of objects at a distance and on moving conveyor belts. In many cases, such as, for example, in the recycling of automotive scrap, the geometry of the objects to be analyzed can vary, and surface coatings can be present. Independently on the specific application, the problems associated to the on-line analysis are often similar; in particular, the LIBS signal are in general quite low and variable, so it is important the use of the most advanced experimental strategies for improving the signal/noise ratio. At the same time, robust and fast methods must be used for the analysis of the thousands of spectra that can be accumulated in few minutes in the on-line applications of LIBS.

In this communication, we discuss the application of the Laser-Induced Breakdown Spectroscopy (LIBS) technique to the industrial projects in which our Laboratory is involved, to illustrate how the above-mentioned problems can be faced and successfully solved.

**Keywords:** Laser, Plasma, Process Monitoring, Spectroscopy

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

**Methodology Code:** Atomic Spectroscopy/Elemental Analysis
Laser ablation is a green, disruptive technology to replace dissolution required for solid sample chemical analysis. Laser ablation is the sampling process; a pulsed laser beam removes mass from a sample for subsequent analysis. The ablated mass is transformed into a luminous optical plasma that condenses to a fine aerosol. The aerosol is transported to an Inductively Coupled Plasma Mass Spectrometer (ICP-MS) for sensitive and precise isotopic analysis. The next advance in this technology is to open up the periodic table of elements that can be detected with each laser pulse by combining optical (LIBS) with particle (ICP-MS) detection. Optical emission from the laser plasma monitored simultaneously with the ICP-MS detection provides complementary elemental and isotopic analysis of the sample.

LIBS is ideal for analyzing light elements, for example Li, Be, S, C, O, N, H and the halogens; elements that are difficult or impossible with the ICP-MS. In addition LIBS is good for major concentrations which again are problematic in the ICP-MS, expanding the dynamic range of analysis. These two detection modalities complement each other. To go one step further is to measure the isotopes of these light elements and halogens. However, splitting in atomic and ionic spectra from isotopes in atmospheric pressure laser plasmas is generally small and poorly resolved. Our new technology LAMIS (Laser Ablation Molecular Isotopic Spectroscopy) measures isotopes in laser plasmas at atmospheric pressure from molecular emission band spectra in addition to atomic and ionic line spectra. We developed LAMIS to date by measuring B, C, H, D, Sr and other isotopes. We demonstrated low percent levels for sensitivity and have experimental plans to meet ppm levels. For some isotopes, we have achieved < 0.1% precision. The talk will describe the simultaneous measurement of photons (LIBS, LAMIS) with particles (ICP-MS) for detecting of every element/isotope in a sample with a single laser pulse.
LIBS is currently a subject of great interest in spectroscopy because it is well suited for field portable applications that cannot be addressed by conventional analytical methods. Since the invention of the laser in the sixties, a few instruments based on LIBS have been developed but have not found widespread use. In the last decade, there has been a renewed interest in the method for a wide range of applications. A bibliographic study around the LIBS literature shows clearly the growing number of LIBS application areas.

The adoption of portable and handheld instruments is gaining momentum, as the need for quick and on-the-spot analysis is increasing. That importance is due to their ability to support online analysis of samples where it is difficult to carry benchtop instruments. Their key application areas include drug identification, food inspection, environmental application, metallurgy and the defense sector. Portable instruments are gaining more importance especially in the food and healthcare industries. Higher growth is expected in many regions of the world, where the need for safety in drugs, food, and environmental health is increasing. Portable instruments do not require the use of reagents, do not produce analytical waste, are fast and allow on-the-spot analysis and they have increasing features and functionality. Thus, portable instruments are good candidates to respond to the growing needs for in-situ analysis and they also contribute to keeping the environment green.

The portability aspect of the LIBS devices constitutes a major asset of this evolving technology. However, the level of portability needed for some applications imposes some restrictions on the choice of many of the core components used in a low cost LIBS handheld sensor unit. In the presentation, we will discuss the related challenges, the comparison with established conventional techniques and the LIBS future related to the discovery of new enabling tools and components.

Keywords: Laser, Plasma, Portable Instruments, Spectroscopy
Application Code: Process Analytical Chemistry
Methodology Code: Atomic Spectroscopy/Elemental Analysis
In this talk, we will present the various Laser Induced Breakdown Spectroscopy (LIBS) application to food sciences. In first application, LIBS technique was used to identify and compare the presence of major nutrient elements in organic and conventional vegetables. Different parts of cauliflowers and broccolis were used as working samples. Both univariate and multivariate analysis were performed for the comparison of these organic and conventional vegetable flowers. Principal component analysis (PCA) was taken into account for multivariate analysis while for univariate analysis, the intensity of selected atomic lines of different elements and their intensity ratio with some reference lines of organic cauliflower and broccoli samples were compared with those of conventional ones. In second application, wheat flour tortillas of different colors and composition were studied with the aid of LIBS. Due to its simplicity, fast response, little or no sample preparation, LIBS is presented as an effective tool for rapid in situ sample analysis of food samples. Spectral properties of the Ca, Na and K elements such as peak intensities, intensity ratios, and area under spectral lines were analyzed. Tortillas manufactured by Gruma Corporation and Mexamerica, of brown, orange, green and white colors as well as homemade tortillas were studied in this work and the results from both dried and undried samples is presented. In third application, LIBS technique is used to compare the various types of commercial dairy milk powder products. LIBS was investigated for the determination of the elemental composition of soy, rice, and almond milk powders. The atomic emission from Ca, P, Zn, K, Na, and Mg lines and the molecular emissions from C2 and CN bands observed in LIBS spectra of dairy milk powders composition were compared. Some of the other LIBS application to food sciences will also be summarized.

**Keywords:** Atomic Emission Spectroscopy, Atomic Spectroscopy, Food Science, Plasma

**Application Code:** Process Analytical Chemistry

**Methodology Code:** Atomic Spectroscopy/Elemental Analysis
Atomically precise, nanometer-scale, ligand-stabilized noble metal clusters have emerged in recent years as a novel form of nanoscale matter with potential applications in molecular electronics, optics, sensing, drug delivery and biolabeling (1). Tremendous advances have been achieved in understanding their stability and structure due to contributions from synthetic work, X-ray crystallography, NMR spectroscopy and density functional theory computations. The general features of their electronic structure can be understood surprisingly well from simple concepts, particularly from the so-called “superatom model” that accounts for the delocalized metal sp-electrons in the metal core (2). The organic ligand layer facilitates chemical functionality and imparts in many cases chirality. Some recent advances in unraveling the structure and properties of these novel nanomaterials composed of gold, silver, or their intermetallics are discussed (3-7), and a novel application for site-specific conjugation to enteroviruses for TEM imaging is demonstrated (8).

References:

Keywords: Material Science, Nanotechnology
Application Code: Nanotechnology
Methodology Code: Surface Analysis/Imaging
The high curvature and chemically heterogeneous surfaces of semiconductor nanocrystals (quantum dots) make their characterization a challenge. Quantitative knowledge of their surface chemistry is, however, critical to their optical and electronic properties. This talk includes discussion of techniques such as measurements of rate and yield of interfacial electron transfer (photoinduced and spontaneous) and interfacial energy transfer, vibrational spectroscopy, and nuclear magnetic resonance to count, identify, and determine the binding geometries of organic molecules adsorbed to quantum dots.

**Keywords:** Luminescence, Magnetic Resonance, Nanotechnology, Surface Analysis

**Application Code:** Nanotechnology

**Methodology Code:** Molecular Spectroscopy
Catalytic hydrogenations are critical steps in many industries including agricultural chemicals, foods and pharmaceuticals. Typical heterogeneous hydrogenation catalysts involve nanoparticles composed of expensive noble metals or alloys based on platinum, palladium, rhodium, and ruthenium. We demonstrated for the first time how single palladium atoms can convert the otherwise catalytically inert surface of an inexpensive metal into an ultraselective catalyst. High-resolution imaging allowed us to characterize the active sites in single atom alloy surfaces, and temperature programmed reaction spectroscopy to probe the chemistry. The mechanism involves facile dissociation of hydrogen at individual palladium atoms followed by spillover onto the copper surface, where ultraselective catalysis occurs by virtue of weak binding. The reaction selectivity is in fact much higher than that measured on palladium alone, illustrating the system’s unique synergy.

Our single atom alloy approach may in fact prove to be a general strategy for designing novel bi-functional heterogeneous catalysts in which a catalytically active element is atomically dispersed in a more inert matrix. Very recently we demonstrated that this strategy works in the design of real catalysts. Palladium/copper nanoparticles containing <2% palladium exhibited highly selective hydrogenation of phenylacetylene under realistic reaction conditions and platinum/copper nanoparticles perform the industrially important butadiene hydrogenation at lower temperature using just 1% platinum. Our scientific approach allows one to parse out the minimal reactive ensembles in an alloy catalyst and provide design rules for selective catalytic nanoparticle. From another practical application standpoint, the small amounts of precious metal required to produce single atom alloys generates a very attractive alternative to traditional bimetallic catalysts.

Keywords: Imaging, Metals, Nanotechnology, Single Molecule
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Surface Analysis/Imaging
In has been recognized for some time that strong interaction of electromagnetic fields (e.g., light) with plasmonic nanomaterials offers opportunities in various technologies that take advantage of photophysical processes amplified by this light-matter interaction. More recently, it has been shown that in addition to photophysical processes, optically excited plasmonic nanoparticles can also activate chemical transformations directly on their surfaces. This potentially offers a number of opportunities in the field of selective chemical synthesis.

I will discuss our findings that plasmonic silver nanoparticles, optically excited with low intensity visible light, exhibit direct photo-catalytic activity. I will discuss underlying mechanisms associated with these phenomena. We propose that this new family of photo-catalysts could prove useful for many heterogeneous catalytic processes that cannot be activated using conventional thermal processes on metals or photo-catalytic processes on semiconductors. I will show an example of such a process.

2. D. B. Ingram, S. Linic, JACS, 133, 5202, 2011

Keywords: UV-VIS Absorbance/Luminescence
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Chemical Methods
Plasmonic nanomaterials are promising candidates for solar-driven catalytic devices, as they interact strongly with light and are known to be capable of driving energetically unfavorable chemical reactions. However, significant questions remain as to the mechanism of action of a plasmonic photocatalytic system, in part because of difficulties in characterizing the photo-induced dynamics and interactions between the nanoparticle surfaces and proximal molecular species. In particular, the impact of hot carriers on driving photochemistry, including fundamental properties such as their yield and lifetime, is currently poorly defined.

Here we use ultrafast surface-enhanced Raman spectroscopy, a pump-probe technique designed to follow the molecular response of molecules adsorbed on plasmonic nanomaterials, to investigate the response of the coupled molecule-plasmon system to light. By tracking the transient Raman frequency evolution, we can determine the degree of charge transfer and molecular structural change following plasmon excitation. Here we discuss ultrafast Raman studies on both monometallic and heterometallic antenna-reactor complexes. For silver colloidal samples, we find a strong correlation between sites on the nanoparticle surface which generate hot electrons and those which drive photochemistry. For heterometallic nanomaterials, we investigate the role of transient molecular intermediates in promoting selectivity in photocatalysis. Our work on the fundamental mechanism of how the nanomaterial surface interacts with nearby molecular species on the ultrafast timescale should ultimately lead to rational design of plasmon-driven photocatalytic systems.

This material is based on work supported by the Air Force Office of Scientific Research under AFOSR Award No. FA9550-15-1-0022.

Keywords: Nanotechnology, Raman Spectroscopy, Surface Enhanced Raman Spectroscopy, Ultra Fast Spectroscopy

Application Code: Nanotechnology

Methodology Code: Vibrational Spectroscopy
Chronic neurochemical monitoring is a fundamental step towards understanding the neural basis of human behavior. Studies have shown that abnormal neurochemical signaling is a primary cause of many brain disorders. To treat such disorders, it is important to understand neurochemical dynamics over the long-term. Carbon fiber microelectrodes (CFM), the current gold standard electrode for neurochemical studies, offers high chemical sensitivity at the expense of increased surface fouling. This limits CFM’s utility during chronic measurements. Emerging carbon nanomaterials have spurred renewed interest in investigating new electrode material technology. Among them, boron-doped ultrananocrystalline diamond (UNCD) with its intrinsically high chemical inertness, electrochemical and dimensional stability, could emerge as an ideal chemical sensing electrode. In this talk, we present recent progress in the development of UNCD microelectrodes for chronic dopamine detection. Long-term electrode surface stability and changes in their electrochemical properties will be reported. Electrode reactivation strategies to maintain chemical sensitivity and selectivity towards dopamine will also be presented. Finally, the development of electrochemical models to explain the progression of surface fouling using impedance techniques will be elucidated in some detail.

Keywords: Electrode Surfaces, Neurochemistry, Sensors, Voltammetry
Application Code: Neurochemistry
Methodology Code: Sensors
Here we report the development of a nanotechnology based electrochemical method for rapid profiling of protease activities through monitoring the proteolytic kinetics of ferrocene (Fc)-labeled-peptides that are immobilized at the exposed tips of nanoelectrode arrays (NEAs) fabricated with vertically aligned carbon nanofibers (VACNFs). Using alternating current voltammetry (ACV), an enhanced AC current signal of Fc can be clearly extracted and monitored in time, reflecting the proteolytic activity of specific proteases that cleave the peptides. The detection of the activity of a cancer related protease, cathepsin B, has been demonstrated with purified recombinant cathepsin B in buffer solutions and spiked in human tissue lysates. An algorithm based on heterogeneous Michaelis-Menten model has been developed. This algorithm has been further validated in detecting cathepsin B activities in complex breast cell lysates. Four types of breast cells have been tested, including normal breast cells (HMEC), transformed breast cells (MCF-10A), breast cancer cells (T47D), and metastatic breast cancer cells (MDA-MB-231). The detected protease activity was found increased in cancer cells, with the MDA-MB-231 metastatic cancer cell lysate showing the highest cathepsin B activity. The equivalent cathepsin B concentration in MDA-MB-231 cancer cell lysate was quantitatively determined by spiking recombinant cathepsin B into the immunoprecipitated MDA-MB-231 lysate and the HMEC whole cell lysate. Clear inhibition to the cathepsin B activity was observed after adding specific inhibitors to the complex tissue lysates. The results illustrated the potential of this technique as a portable multiplex electronic device for cancer diagnosis and treatment monitoring through rapid profiling the activity of specific cancer-relevant proteases. It can be also used as an analytical platform for rapid screening of effective protease inhibitors as potential anti-cancer drugs.

**Keywords:** Biomedical, Chemically Modified Electrodes, Enzyme Assays, Nanotechnology

**Application Code:** Biomedical

**Methodology Code:** Electrochemistry
Graphene and carbon nanotube (CNT) are two important members of nanocarbon materials. Both of them exhibit distinct properties that are highly attractive for biosensing and bioimaging, such as high electrical conductivity, large surface area, and excellent ability to quench fluorescence. In our group, we have successfully prepared functionalized CNTs, graphene and graphene oxide containing a variety of features with different specificities, and further utilized them for biosensing, bioimaging, and many other applications as well as fundamental research. In this talk, I will give an overview of recent works done in my laboratory and use a few examples to explain how to use the unique property of CNT and graphene for different applications in biosensing and bioimaging.
Carbon materials have been used over the years in energy storage and conversion, chemical analysis and chromatographic separations. The widespread interest in carbon stems from its low cost, mechanical strength, chemical stability in a variety of environments and diversity of surface chemistry. Nonetheless, advances in the fundamental understanding of the structure of electrified interfaces formed at various carbon materials and how this structure affects adsorption, charge-transfer kinetics and redox reaction mechanisms remain to be gained. This is particularly true for the novel sp3 (diamond) and sp2/sp3 (tetrahedral amorphous carbon) electrodes. A more complete understanding of structure-property relationships will serve as the foundation for the next-generation of electrochemical sensors and detectors with these carbon materials.

We are studying the structure-property relationships of boron-doped nanocrystalline diamond and nitrogen-incorporated tetrahedral amorphous carbon (ta-C:N) thin-film electrodes. The diamond-like carbons, of which ta-C belongs, is a class of materials that has been comparatively unstudied in terms of their electrochemical properties and electroanalytical performance. These are composite materials consisting of a mixture of sp2 and sp3-bonded carbon. Impurities can be incorporated during growth (e.g., N) further adding to their complex structure. These films typically possess 40-60% sp3-bonded carbon. ta-C has been widely used as a protective coating due to its hardness, high wear resistance and low coefficient of friction. The growth temperature for ta-C is usually from 25 to about 100 oC. The characterization and basic electrochemical properties of these new electrode materials will be reported on and their use in electrochemical detection coupled with FIA-EC and HPLC-EC will be highlighted. Specific examples will include the analysis of electroactive amino acids and pharmaceutical metabolites (estrogenic compounds).

Keywords: Analysis, Bioanalytical, Electrochemistry, Electrodes
Application Code: Pharmaceutical
Methodology Code: Electrochemistry
Carbon Nanomaterial-Enabled Microsensing Technologies

In Vivo Carbon Nanotube Sensors

Single Wall Carbon Nanotubes have been introduced to multiple animal models in the past, but the in vivo use of carbon nanotube based sensors involves specialized delivery mechanisms so that the sensing modality, created by wrapping the carbon nanotube with specific polymers, is not impeded in the animal. In this work multiple methods for in vivo carbon nanotube sensor delivery are highlighted, including various hydrogel platforms as well as the delivery of a liquid sensor. The techniques utilized for the in vivo delivery of carbon nanotube sensors can be extended to be used by various small sensors and in various animal models.

Keywords: Biosensors, Fluorescence, Nanotechnology, Near Infrared
Application Code: Nanotechnology
Methodology Code: Sensors
Traceability within the agriculture and agro-foods industry is the cornerstone of food security. This is largely due to the increasingly complex sources of agricultural produce through the globalisation of agricultural trade and the threat of disease, contaminated produce and fraudulent labelling of produce. Governments and consumers are demanding more stringent labelling and proof of traceability (or provenance) and authentication of whether the produce is imported or domestic in order to promote confidence in the agro-food industry and national brands.

While the bulk of the traceability programs involve farm-to-table tagging of produce through bar codes and lot numbers, multi-isotopic analyses of produce and their source areas also provide a powerful and complementary tracing technique that can validate the provenance and authenticity of agricultural produce. The multi-isotopic approach involves combining both stable (hydrogen, oxygen, carbon, nitrogen) and radiogenic isotope (Strontium) systems. The stable isotopes of hydrogen and oxygen of the water within agricultural produce reflect the climate and information pertaining to the geographical context in which the produce is grown. Carbon and nitrogen isotopes provide evidence of diet (cattle) and fertilization. Strontium isotopes reflect the geology of the growing area and together with the oxygen and/or hydrogen isotopes provide constraints on the growing region. The isotopic analyses thus provide a geochemical characterization of the terroir concept. Examples of the advantages and limitation of the approach are demonstrated for cheese and wine from the province of Quebec, Canada.

Keywords: Agricultural, Food Safety, Food Science, Isotope Ratio MS
Application Code: Agriculture
Methodology Code: Mass Spectrometry
Our team has developed RF-based sensors for physical, chemical and biological sensing and have tailored the sensors for a variety of different food quality applications. We tailor different components of the sensor including the antenna, tag and the intrinsic design coupled with chemical or biological films in certain instances to simultaneously measure several parameters of the complex impedance. Our approach is data-rich owing to the multitude of parameters measured at each data point, resulting in the potential for multivariate data analysis. We will discuss different signal processing methods based on full-spectral and feature-selected analysis of impedance responses of individual sensors and how they are applied to increase the sensitivity and selectivity of our sensors. We will also highlight development of the multi-generational RF reader and the current state of the art offering developed by KemSense.

This work has been supported in part by GE Fundamental Research and KemSense Funding and by the National Institute Of Environmental Health Sciences (Grant 1R01ES016569-01A1).

Keywords: Agricultural, Food Safety, Food Science, Sensors
Application Code: Food Safety
Methodology Code: Sensors
This presentation will focus on the advantages of Raman microspectroscopy and its applicability for use in solving food industry challenges. The benefits of Raman spectroscopy are numerous and include the ability to non-destructively analyze samples in-situ, without any interference from water, while obtaining results in real-time without modification of the sample. Traditional microscopy utilizes stains to help identify protein, fat or carbohydrates in food samples; however, Raman microspectroscopy allows the user to not alter the sample or the ingredients location while still being able to detect and identify each of these ingredients at the same time. The inherent advantage of Raman microspectroscopy is that one can characterize the vibrational modes of all the ingredients being used in the product by evaluating neat samples first, and then looking for conserved vibrational modes in the complex finished food product itself. A great example of the viability of this technique is that certain ingredients that cannot be stained for, such as flavor compounds or additives, can still be identified within the sample since the spectroscopic data can be analyzed by looking for key vibrational modes that are characteristic of these ingredients.

The versatility of this methodology is just starting to be realized due to the advancements in laser technology, detection systems, mapping capabilities and software advancements that allow for collecting and interrogating rather large sample areas in extremely short times. A thorough discussion of Raman microspectroscopy and its advantages will be presented along with a number of examples of how this technology is being used in the food industry.

Keywords: Food Science, Microspectroscopy, Raman Spectroscopy, Spectroscopy
Application Code: Food Science
Methodology Code: Molecular Spectroscopy
There are many applications of Nuclear Magnetic Resonance (NMR) Spectroscopy in the area of analysis of food and food related products. A general overview of the most common uses of NMR in food analysis as well as several specific applications will be presented with possible advantages and limitations evaluated throughout. In particular, the following techniques will be discussed with example applications: quantitative analysis using NMR (qNMR) using both proton detected (more common) and carbon-13 detected (less common) experiments, "iso-tagging" (derivatization of an analyte with an NMR-active, isotopically labeled group) for characterization and quantification, and chemometric analysis of proton NMR spectra as "fingerprints", i.e. without direct assignment of any peaks.

**Keywords:** Food Contaminants, Food Identification, Food Safety, NMR

**Application Code:** Food Identification

**Methodology Code:** Magnetic Resonance
Translational Microfluidic Platforms for Clinical Diagnostics

Electrophoretic Cytometry: High Selectivity Measurement of Cell-to-Cell Variation in Protein Signaling

Dysregulation of signaling pathways is a hallmark of diseases including cancer. Cell-to-cell variation in this dysregulation is a critical aspect of disease development and progression. While single-cell resolution genomic and transcriptomic tools are advancing, these nucleic acid measurements do not directly measure protein-mediated signaling including dynamic post-translational protein modifications, protein localization, and protein-protein interactions.

Powerful single-cell protein analysis tools do exist, yet the analytical specificity of immunoassays is dictated by immunoreagents which are notorious for sub-optimal performance. We recently reported on a microfluidic approach to achieve single-cell resolution western blotting, thus enabling direct measurement of proteins in single cells when high selectivity. This presentation will discuss both fundamental and applied aspects of the targeted proteomics tools. In fundamentals, we will detail how a combination of separations science, microfluidics, and polymer formulation contributions offers a single-cell western blot with high throughput (>6,000 concurrent single-cell protein assays). In applied aspects, the presentation will highlight biological studies made possible by high selectivity protein measurements, including as related to drug resistance development in breast cancer, circulating tumor cells as indicators of cancer disease state, and as a tool to monitor sub-cellular localization of proteins as is relevant to IRES trans-acting factors. Looking forward, we see single-cell resolution targeted proteomics as an important complement to nucleic acid measurements and discovery proteomics.

Abstract Text

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Abstract Text

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Keywords: Bioanalytical, Clinical Chemistry, Electrophoresis, Lab-on-a-Chip/Microfluidics
Application Code: Biomedical
Methodology Code: Microfluidics/Lab-on-a-Chip
During the past decade, our group has used microfluidic technologies to mimic the role of the red blood cell and other bloodstream components in the circulatory system. These studies have often been informed efforts branching into other, non-fluidic based assays investigations. Here, I will describe one such effort where our group has used properties of the red cell as the basis for a blood-based lab test for identifying patients with multiple sclerosis (MS). In this study, whole blood from subjects with MS, non-MS neurologic diseases, and healthy controls was centrifuged to isolate erythrocytes. Following the addition of exogenous C-peptide, the supernatant was assayed for remaining C-peptide using an enzyme linked immunosorbent assay (ELISA). Our study included subjects with MS (n=86), other non-MS neurologic diseases (OND n=75), and healthy controls (n=39). The average C-peptide bound to erythrocytes in MS samples (3.51 ± 0.59 picomoles) was significantly higher than non-MS subjects (2.23 ± 0.51 picomoles; p<0.001) and healthy controls (1.99 ± 0.32 picomoles; p <0.001). During these pre-clinical studies, our group has implemented many tools, many statistical tools, with which we were not previously familiar, to demonstrate diagnostic utility. For example, a receiver-operator characteristic (ROC) curve generated from the ratio of the sensitivity to 1-selectivity resulted in an area under the curve of 0.97. Using the ROC curve, a cutoff of 3.04 picomoles of C-peptide uptake was determined as the optimal threshold for the diagnostic odds ratio. This cutoff point was also used as the point of optimum sensitivity (98.3%) and specificity (89.5%). In this presentation, we will also discuss our group’s exposure to strict control studies, validation studies, and calibration protocols.

Keywords: Biological Samples, Biomedical, Biotechnology, Neurochemistry
Application Code: Clinical/Toxicology
Methodology Code: New Method
Cellular and molecular assay, especially in the study of phenotype-genotype correlations at the single-cell level, are critical for the understanding of intratumor heterogeneity and identification of cancer phenotype-related genes, new cell subsets, and assist in cancer prevention, diagnosis, and therapy. Traditional technologies for single-cell manipulation and analysis are plagued by operational complexity, limited efficiency, and low-throughput. Integrated microfluidic devices have become a robust technique for single-cell manipulation. By the rational design of microfluidic platforms, we can achieve rapid and high-throughput cellular and molecular assays, including 1) single cell based analysis such as Block-Cell-Printing for live single-cell printing, Single-Cell Pipette for convenient single-cell isolation, and yeast chip for high-throughput analysis of yeast replicative aging, 2) double cells based assay such as vertical cell pairing for high-resolution imaging of the immunological synapse, and 3) CRISPR/Cas9 based genome editing for hard-to-transfect cells.
Precision medicine seeks to match patients to appropriate therapies that optimize clinical outcome from molecular signatures of their disease. These molecular signatures can be secured from circulating markers found in blood, which represents an exciting diagnostic scenario because of the minimally invasive nature of securing these markers and the plethora of marker types found in blood including biological cells (Circulating Tumor Cells, CTCs), cell-free molecules (cell-free DNA) and/or nanoscale vesicles (exosomes). Unfortunately, many of these blood-borne markers have not been effectively used in clinical practice primarily due to the fact that disease-associated blood markers are a vast minority in a mixed population making them difficult to find and analyze due to deficiencies in the technologies used for their isolation and systems that can determine the clinically actionable molecular signatures they harbor. To address this deficiency, we are generating innovative microfluidic systems for selecting circulating markers from whole blood and determining the presence/absence of disease-specific molecular signatures to guide therapy for a patient. In this presentation, we will provide a discussion on our work to affect the delivery of microfluidic systems into the clinic for isolating and analyzing circulating markers and the clinical decisions they produce. As examples, circulating markers for guiding therapeutic decisions in colorectal and pancreatic cancers will be discussed using circulating tumor cells (CTCs) as the markers.

Keywords: Biomedical, Biosensors, Biospectroscopy, Biotechnology
Application Code: Biomedical
Methodology Code: Microfluidics/Lab-on-a-Chip
Insights into epigenetics and chromatin dynamics have profoundly affected our understanding of biological processes including development, aging, complex diseases and oncogenesis, providing a more comprehensive view than could be ascertained by considering information from genetic or gene expression studies alone. Detection of genome-associated protein complexes, as well as histone post-translational modifications and positioning, are crucial for developing of new diagnostic and therapeutic strategies. However, the methods utilized in the research laboratory are not at present readily translatable to clinical applications. We are developing a suite of droplet microfluidic components that can be integrated into a robust and automated platform supporting chromatin immunoprecipitation and histone footprinting, followed by sequencing. The ability to perform these assays with high reproducibility and with minimal sample input requirements is poised to enable the clinical realization of personalized epigenetics.

Keywords: Biomedical, Clinical Chemistry, Genomics, Lab-on-a-Chip/Microfluidics
Application Code: Biomedical
Methodology Code: Microfluidics/Lab-on-a-Chip
Before treating the subject of how to be successful in a career, we must first define the meaning of success. While everyone has different hopes for a career, there are some common characteristics shared by all. Methods for achieving success are also varied; but again, certain skills, actions and behaviors can optimize one’s chances of achieving success. Over the past 40 years, I have enjoyed what I would consider to be a successful career for me, during which time I have attempted to convey some insights to my approximately 100 talented graduate students and postdoctoral researchers about how they could also enjoy success. From my viewpoint, some key considerations include work ethic, determination, passion for the work, communication skills, planning and organizational skills, self-confidence, resourcefulness, flexibility, and interpersonal skills. It is important to have an overall feeling of accomplishment and well-being, although some stress and anxiety is not all bad. In this presentation, I will attempt to illustrate many of these points with personal experiences.

Keywords: Capillary GC, Capillary LC, Mass Spectrometry, Teaching/Education
Application Code: General Interest
Methodology Code: Education/Teaching
CACA - How to be Successful in Your Career

The Importance of Choosing a Career Doing Something You Really Like

This talk will start with a brief overview of my personal career experiences focusing on the importance of picking a career that involves doing something that you really like. Often one may be motivated to select a career based on the expected salary but it’s difficult to feel happy and fulfilled over the course of a career doing something that you don’t truly enjoy. At the same time it’s also important to pick a career that has staying power. It’s better to pick a career involving a new and exciting technology not only because it will likely be more fulfilling but also because it’s a lot more likely that such a technology, at least in some form, will still be around at the end of your career. All of the above factors will be discussed in detail, using personal experience to illustrate the pros and cons of potential career choices.

Abstract Text

Analysis, Chromatography

Other

Liquid Chromatography
I have worked in both industry and academia for over four decades and want to continue. In both environments I’ve noticed correlations with success that often surprise young scientists and engineers. The culture of numbers and experiments often seems orthogonal to the culture of liberal arts. Many technical people avoid developing the softer skills that matter a lot. Only later do they realize that rising to the top of any organization depends more on listening skills, respect for people, ethics/trust, and team work. Speaking and writing skills are crucial to persuading and leading. They are best developed early and sharpened with frequent practice. In this presentation we will try to have fun, but also encourage new scientists to be very serious about language and culture. This lesson applies to everyone, but especially to scientists now working far from their native country.
### Session # 100  Abstract # 100-4

#### Session Title
CACA - How to be Successful in Your Career

#### Abstract Title
Career Journey from a Scientist to Business Executive

#### Primary Author
Linda De Jesus
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#### Abstract Text
This talk will start with my personal story of choosing a science major during undergraduate, and personal journey evolving from a traditional scientific career path to becoming a business executive. I will share lessons learned along the way and insights gained, including what you will not learn or be told during school and early phase of career. Most importantly, how to stay connected and passionate with the advancement of science. This will be an open discussions on career options and career development opportunities that one will need to consider across various phases of one’s career planning.

**Keywords:** Mass Spectrometry

**Application Code:** Other

**Methodology Code:** Liquid Chromatography/Mass Spectrometry
Nowadays more and more Chinese-American scientists are working in industry and academic after graduate studies. They are continuing to make significant contributions in a variety of fields and play many critical roles in both industry and academia. However, many of them include me face an enormous challenging in the early stage of their career development. Those challenges arise from not only the culture difference between USA and China, but also other uncertainty factors during researches, studies, and visa status that is a prerequisite for working in USA. Although students coming from China have much advanced knowledges in their fields and the related area, they are still far behind American natives, particularly in communication, socialization, and lack of understanding on western culture. Based on my own experiences, I will share some challenges that a Chinese-American scientist may face and how to overcome them.

The presentation will cover several aspects: how to determine a career goal that is best for an individual, how to choose an innovative research that can fully exert your strength and passion, how to improve communication skill that helps your career development, how to be a good team player, how to develop leadership ability and build up a strong networking. Those aspects will mingle with the unexpected opportunity that may completely change your career goal. However, the persistence on pursuit on the goal will eventually lead you to be successful. It is not enough to focus only on your area or research; a scientist has more opportunities to be successful in either industry or academic if he/she is actively involved in the different fields. It is true, as Louis Pasteur’s famous saying, that chance favors only the prepared mind!

Keywords: Carbohydrates, HPLC, HPLC Columns, Mass Spectrometry
Application Code: Genomics, Proteomics and Other 'Omsics
Methodology Code: Liquid Chromatography/Mass Spectrometry
The primary supply of municipal drinking water comes from a variety of water sources such as ground water and surface water. To ensure public safety, the water undergoes disinfection treatments to get rid of potential pathogens. The resulting disinfection by-products, typically present at low concentrations in the presence of high concentration of matrix ions, needs to meet stringent regulatory standards. Ion chromatography with suppressed conductivity detection has been adopted as one of the primary methods for monitoring.

In this talk, we will discuss the development of a multi-dimensional ion chromatography method for achieving the required detection limits for disinfection by-products in drinking water.

The methodology uses a high capacity 4-mm column in the first dimension to separate the analytes from the matrix ions. After separation, the suppressed effluent portion containing the analytes of interest are concentrated onto a concentrator column and subsequently analyzed in the second dimension using a smaller format column (such as 0.4-mm) with a different selectivity, thus resulting in enhanced sensitivity and selectivity. The introduction of capillary scale ion chromatography provides a unique opportunity to further improve the detection limits by using the capillary scale ion chromatography in the second dimension. Specifically we will focus on the application of this technology for haloacetic acid analysis using various water matrices. We will also discuss the quality assurance requirements with respect to the new method for the analysis of haloacetic acids in drinking water.

Keywords: Environmental Analysis, Environmental/Water, Ion Chromatography, Quality Control
It is well accepted to use a suppressor when pursuing ion analysis with an ion chromatograph. The function of the suppressor in ion chromatography is to remove the counter ion to the eluent and convert the eluent to a weakly dissociated form. When pursuing anions analysis with hydroxide chemistry the product of suppression is water and has a low conductivity background and low noise. When pursuing anion analysis using carbonate and/or bicarbonate eluents, the suppressor product is carbonic acid which results in a greater than 10 fold higher background than hydroxide eluents and relatively high noise. In the chemical mode of operation, the noise is not impacted by the suppressed conductivity background; however leakage of the chemical reagent can compromise the operational dynamic capacity of the suppressor and the detection sensitivity.

In this presentation we discuss various configurations of the electrolytic suppressor with the goal of lowering the operational noise when operated with carbonate and /or bicarbonate eluents. Additionally we discuss a new ERS 500e carbonate suppressor design that allowed low noise performance with carbonate and/or bicarbonate eluents. We show results from comparing the performance of the conventional membrane suppressor design with the performance of the new design. In addition we will also discuss a new design of a consumable accessory that allows continuous operation of the carbonate removal device without any external reagents.
The presentation will summarize recent development in applications of ion chromatography (IC) in parenteral IV drug products. The IC methods using anion and cation exchange and ion exclusion mode will be presented. Many of these IC methods have also been described in pharmacopeias.
Many compounds (e.g., amino acids, carbohydrates, and ions) lack a native chromophore or fluorophore, thereby necessitating derivatization for their detection by optical spectroscopy. Electrochemical approaches are often able to detect these compounds with the advantage of not requiring chemical modification prior to detection.

While conductivity is the preferred means of detection for most common ions, other electrochemical approaches have played a critical role as detectors following ion chromatographic separations. For instance, the detection of carbohydrates by Pulsed Electrochemical Detection (PED) is sensitive, reproducible, and direct. PED offers only a limited degree of voltammetric selectivity, and, as such, its true benefit is realized following a chromatographic separation. The highly alkaline mobile phases required for maximum sensitivity of PED at gold electrodes is ideally suited to the use of polymeric ion-exchange phases because of their stability at pH extremes. Similarly, metal ions separated under low pH conditions can be sensitively detected using a modified version of PED, and ions with no inherent electroactivity have been determined using Indirect-PED (InPED). InPED following anion-exchange chromatography has been used to detect biotin, amino acids, and several pharmaceuticals of interest.

In this presentation, recent developments of novel PED approaches will be discussed in detail. Their analytical utility will be highlighted using “real-world” applications. As an example, high performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) and liquid chromatography with mass spectrometry (LC-MS) methods are compared based on their analytical figures of merit to separate a glycan mixture, which is foundational to glycoprotein characterization.

Keywords: Carbohydrates, Chromatography, Detection, Electrochemistry
Application Code: Bioanalytical
Methodology Code: Liquid Chromatography
We describe for the first time efficient suppressed conductometric open tubular ion chromatography (SC-OTIC). For practical eluent concentrations, suppressor active lengths of approximately 1 mm is adequate. In its preferred embodiment, the suppressor consists of a solid polymer ion exchanger block. Two 0.45 mm diameter parallel cylindrical passages are provided – these provide for passage of regenerant water and placement of Platinum wire electrodes. Only a selected portion of each electrode is exposed to direct the field through the active suppression zone. The suppression channel is made by making a crack in the soft polymer block by a needle of a diameter slightly smaller than the outer diameter of the separation/detection capillaries. The suppression channel (crack) runs parallel to and is flanked by the two aforesaid electrode channels through which water is pumped. The suppressor ends of the separation and the detection capillaries are tapered. The tapered ends of the capillaries are inserted into the suppression channel with the tips 0.4-1.1 mm apart. This geometry allows the passage way between the capillary tips to remain open. With a 1 mm long suppression length we were able to suppress 100 mM hydroxide @ 100 nL/min (10 neq/min). With such a suppressor coupled to an AS18 latex coated surface-sulfonated cycloolefin Polymer (COP) capillary column of 28 µm i.d. and using an on-capillary admittance detector (AD), both isocratic and gradient SC-OTIC will be shown. At 170 nL/min (substantially above the Van Deemter optimum), the plate count for fluoride exceeded 70,000 plates/m under isocratic conditions.
Ion chromatography (IC) involves the separation of inorganic and small organic ions. Retention is due to electrostatic attraction between the analyte ion and the oppositely charged sites affixed to the stationary phase. But this simple model becomes more complicated as the eluent ion must displace the analyte ion from the retention sites. Thus, retention is also due to the electrostatic attraction between the eluent ion and the and the oppositely charged sites affixed to the stationary phase. Add in the kosmotropic/chaotropic nature of the analyte and eluent and the hydrophobicity of the analyte ion and stationary phase, and you can see that even simple ion exchange chromatography quickly becomes complex.

Even further there are other modes of IC such as ion exclusion and electrostatic ion chromatography. What do we know about them?

This seminar will provide a primer on the fundamentals of retention and selectivity in the various modes of ion chromatography, and highlight some recent insights into the mechanisms underlying each.

**Keywords:** HPLC, Ion Chromatography, Ion Exchange, Liquid Chromatography

**Application Code:** General Interest

**Methodology Code:** Liquid Chromatography
The past decade we have witnessed significant increases in the operating pressures and speed of analysis of HPLC. Due to the column chemistries, column hardware, and system components (i.e. non-metallic) associated with ion chromatography (IC), it has not experienced the same gains in operating pressures and speed of analysis. Recently the anion- and cation-exchange stationary phases used for IC columns have been produced in 4 µm particle sizes, typically at least 50% smaller than the existing phase. At the same time, IC systems that employ eluent generation, technology that produces the mobile phases needed for IC with only the addition of deionized water to the IC system, were improved to tolerate pressures up to 5000 psi. The smaller particle size resins allow higher resolution separations and this improved resolution can be used to shorten existing methods. This presentation shows how we took four existing common IC applications, the determination of anions in drinking water according to US EPA Method 300.1 Part A, the determination of oxyhalides in drinking water according to US EPA Method 300.1 Part B, the determination of cations in drinking water, and the determination of citrate and phosphate in pharmaceutical products according to the United States Pharmacopeia General Chapter <345>, and shortened the analysis time with equivalent or improved results. The general principles for shortening existing IC methods and developing new methods with 4 µm particle size columns will be discussed.

Keywords: Chromatography, Ion Chromatography, Pharmaceutical, Separation Sciences
Application Code: Environmental
Methodology Code: Liquid Chromatography
We are going to present new approaches to increase the versatility of nanopore-based chemical sensing. The strategies presented depart from the "classical" Coulter counter concept and are mainly based on sophisticated chemical modification of gold nanopores with small molecules and nanoparticles. Thus we will show that nanoparticle-based displacement assays are feasible in chemically modified nanopores and result in an inherent signal amplification that enables the detection of molecular dimension targets by much larger nanopores. Furthermore, we present approaches to turn gold nanopores by their modification with ion-selective peptides into synthetic "protein pores" that feature tunable ion-selectivity.

Keywords: Bioanalytical, Electrochemistry, Ion Selective Electrodes, Nucleic Acids
Application Code: Bioanalytical
Methodology Code: Electrochemistry
We introduce here for the first time the concept of light addressable electrochemical multianalyte ion sensors. A new approach to detect multiple ions at the same time and in a chemically selective fashion has recently been introduced (1): it is a thin layer voltammetric detection approach where peak position serves to identify the ion concentration (strictly, activity). The main promise of this discovery lies in the fact that the ions are all detected at the very same membrane, and therefore at the same location. It is therefore an exciting platform for chemical imaging, where concentration changes can be spatially resolved. One approach, therefore, is to combine this multianalyte chemical approach with a spatially sensitive readout technique.

The group of Justin Gooding at UNSW has recently published on an attractive approach to use light illumination to activate the electrochemistry of a semiconductor electrode surface (2). Light of a suitable wavelength may promote electrons from the valence band to the conduction band of the material, resulting in conductivity only at the spot where illumination occurs.

This talk will elucidate whether the light addressable silicon-based approach can be combined with thin layer polymer coatings to achieve spatially confined electrochemical readouts that can be used to obtain information on the concentration of multiple analytes at the illuminated spot. The approach is very promising because the readout will be limited by the available ion-exchanger sites within the polymeric film, and not by mass transport from the solution to the surface.

References


Keywords: Electrode Surfaces, Ion Selective Electrodes, Sensors
Application Code: Bioanalytical
Methodology Code: Electrochemistry
The presentation discusses mainly solid-contact ion-selective electrodes (SCISEs) prepared with hydrophobic carbon-like polyazulene (PAz)\textsuperscript{1,2} and polypyrrole (PPy)\textsuperscript{3} transducers. It is shown that both pre-polarized transducers prepared with hydrophobic doping ions prevented the water layer formation and improved the $E^{0}$ reproducibility that was in the best case only ±0.7 mV. This surpasses the $E^{0}$ reproducibility of the state of the art conducting polymer based SCISEs.

Other strategies for incorporating barriers in SCISEs is also discussed in the presentation.


Keywords: Ion Selective Electrodes, Material Science, Membrane, Potentiometry
Application Code: Other
Methodology Code: Sensors
Ionophore-Based Chemical Sensors I

Simple Voltammetric Method for the Determination of the Partition and Diffusion Coefficients in Soft Polymeric Membranes

Chemical and biological sensors are often constructed as multilayer systems in which a base electrode is modified by a stack of polymeric membranes. These membranes provide selectivity, improve the detection limit and extend the dynamic response range. However, for adequate sensor response the material properties of these membranes must be optimized. For example the response of the plasticized PVC membrane-coated voltammetric sensor for the anesthetic drug propofol (2,6-diisopropyl phenol) is strongly influenced by the diffusion (Dm) and partition (Pma) coefficient of propofol in the PVC membrane, i.e., the determination of Dm and Pma is essential for the sensor optimization. Similarly, the measurements of the diffusion and partition coefficients are equally important in other applications such as: ion-selective electrodes, pharmaceutical screening, drug delivery devices, and waste water treatment.

In our contribution we discuss the unique advantages and limitations of a simple voltammetric method for the determination of diffusion and partition coefficients in soft polymeric membranes, like plasticized PVC membranes and polyvinyl alcohol-based hydrogels. To demonstrate the utility of the voltammetric method a planar electrochemical cells with carbon fiber working electrodes was used to determine the Dm and Pma values of ferrocene carboxylic acid, propofol and p-acetaminophenol in 2-nitrophenyl octyl ether, bis(2-ethylhexyl) sebacate, and 1-octanol plasticized PVC membranes cast with 1/2, 1/4, or 1/8 PVC/plasticizer ratios. Dm increased with decreasing plasticizer viscosity and with decreasing PVC/plasticizer ratio, in agreement with the Stokes-Einstein model. Pma values strongly depend on the lipophilicity of the analyte and the plasticizer.

Keywords: Biosensors, Electrodes, Membrane, Voltammetry
Application Code: Biomedical
Methodology Code: Electrochemistry
Current state-of-the-art ion-sensing membranes (ISMs) are passive sensing platforms (i.e. non-activatable), have low sensitivity (low signal-to-noise ratio), and lack the robustness required to operate in biological systems. Proposals to overcome these limitations include, transforming ISMs into activatable and controllable platforms by integrating photoactive compounds, such as photoacids generators or spiropyrans. However, these compounds either irreversibly dissociate protons, require external source of protons or are activated by ultraviolet (UV) light, which has low cell penetrating ability, causes cellular damage, and photodegradation of the photoactive compound. To surmount these barriers, our research group had propose the use of mPAHs coupled to ISMs. The properties of mPAHs are ideal for ion-exchange processes since their photodissociation is reversibly, long-lived and activated by visible light. However, we now need mPAHs with increased fluorescence quantum yields, higher pKa’s, and longer activation wavelengths to deliver effective activatable ion-sensing platforms for biological environments. Therefore, here we will show a systematically study structure-property relationships of mPAHs to expand the fundamental understanding of these compounds and broaden their application to ion-sensing for biological environments. This transformative research represents a true breakthrough in activatable and controllable ion-sensing technology.


Keywords: Bioanalytical, Sensors
Application Code: Bioanalytical
Methodology Code: Sensors
Conducting polymers have been attracting research attention in context of sensors for many years now; however, mostly in the format of layers used for electrochemical sensors. On the other hand nanospheres of conducting polymers offer possibility of exploring attractive analytical properties of these materials together with benefits of nanoscale probes. For some polymers, e.g., polythiophenes, fluorescence properties depend on the state of the polymer. Moreover these materials can be easily obtained in the form of nanoparticles using nanoprecipitation. The compatibility of these materials with ionophores, opens a possibility to obtain nano-optodes. In contrary to conventional optodes, the proposed sensors do not require presence of pH-sensitive dyes and show turn-on, optical responses with a linear dependence of emission intensity on the logarithm of analyte contents in a broad range (a few of orders of magnitude) [1].

For electrochemical sensors it is vitally important that nanoparticles of conducting polymers are characterized with uncompromised electrochemical activity, which is usually difficult to achieve using conventional synthetic approaches. The recently proposed by us [2] novel templateless method of conducting polymers nanoparticles preparation yields formation of nanospheres of highly active surface, free from modifications and forming stable suspensions in aqueous phase. Thus obtained structures are characterized with high electrochemical activity and can be used to construct electrochemical or optical sensors, moreover they can be applied either as transducers or receptors. They can be used on non-conducting surfaces, to result in electrochemically active layers. On the other hand, nanospheres sensitized with fluorescent dyes can be also used to follow changes in ionic analyte concentration in solution in emission mode.


Keywords: Electrochemistry, Electrodes, Fluorescence, Nanotechnology
Application Code: Process Analytical Chemistry
Methodology Code: Electrochemistry
The primary focus of this research is the investigation of the mechanism of biofouling in current fluorous phase ion-
selective electrode (ISE) systems. With prolonged exposure to biological samples, potentiometric measurements using
conventional polymeric-membrane ISEs exhibit a breakdown of selectivity and response. Therefore, extensive washing
procedures and frequent recalibrations are needed for many clinical and biological applications. Initial work with fluorous
phase ISEs has shown significant improvements in selectivity and limits of detection over conventional polymeric-
membrane ISEs. Moreover, experiments with fluorous pH electrodes have shown that long term serum exposure does
not affect the electrode selectivity but stirred serum solutions appeared to cause a transient EMF drift. To explore this
effect more systematically, a potentiometric stir tests was developed. Both conventional polymeric membrane and
fluorous membrane electrodes were exposed to solutions stirred intermittently. Both types of membranes exhibited an
EMF response to stirring when exposed to of 10% v/v solutions equine blood serum but not when exposed to simple
electrolyte solutions. The transient potentiometric response depends on the hydrophobicity of the ionic sites
incorporated into the ISE membranes; specifically, a lower hydrophobicity results in a larger effect of stirring on the EMF.
For the fluorous electrodes, synthesis of a more hydrophobic ionic site and its use along with fluorophilic H+ ionophores
successfully mitigated the effect of sample stirring on the emf. The use of a fairly simple phase boundary model confirms
that the effect of stirring is caused by loss of ionic sites into the serum containing sample. This is to the best of our
knowledge the first report that shows that the leaching of ionic sites into serum samples can be directly observed by
potentiometric monitoring of the effect of sample stirring on the EMF.

Keywords: Biomedical, Electrochemistry, Electrode Surfaces, Ion Selective Electrodes
Application Code: Bioanalytical
Methodology Code: Electrochemistry
Using inexpensive sensors to quantify a broad range of biomacromolecules is a fascinating research filed because of its huge potential to revolutionize clinical diagnostics, disease monitoring and other related fields. Taking advantages of the wide utility and low cost of the polymeric membrane ion-selective electrodes (ISEs), we demonstrate here a novel sensing platform to use such sensors to detect biomacromolecules. Despite their great promises, to date it is still a challenge to achieve potentiometric detection of biomacromolecules without chemical labels since a receptor-biomacromolecule interaction usually does not yield a measurable potential signal. Here we address this challenge by combination of potentiometry with the surface imprinting technique. The proposed sensing strategy is based on the surface blocking mechanism for which the recognition reaction between the surface imprinting polymer and biomacromolecules can block the flux of the marker ion from the sample solution to the sensing membrane, thus inducing the membrane potential change. Experimental results show that biomacromolecules such as proteins and cells can be detected by using ISEs without chemical labeling.
Analysis of Pharmaceutical Ingredients by GC (Half Session)

**GC-FID Method for High-Throughput Analysis of Residual Solvents in Pharmaceutical Drugs and Intermediates**

Gas Chromatography-Flame Ionization Detection (GC-FID) analysis for quantitation of residual solvents in drugs and synthetic intermediates is one of the most important and frequently used tests in the pharmaceutical industry. However, as currently practiced, the technique requires significant sample preparation time, in addition to having a very poor ‘green factor’ (Analytical Method Volume Intensity, or AMVI). In this study, a simple and fast protocol using multi-solvent standard mixtures combined with a seven minute universal GC-FID method (using either He or H2 as carrier gas) and Empower™ data analysis is presented. We demonstrate that standard mixtures containing solvents commonly used in process chemistry workflows can be stored in crimped HPLC vials at 10 °C for at least 31 months. The 31 months stability data showed over 97% recovery for all 25 solvents, with overall relative standard deviation below 5%. Our approach simplifies tremendously the tedious task of residual solvent quantitation, resulting in significantly less labor, greater reliability, faster time to result and at least a 290 fold reduction in solvent consumption and hazardous waste disposal.

**Keywords:** Gas Chromatography, High Throughput Chemical Analysis, Pharmaceutical, Process Analytical Chemis

**Application Code:** Pharmaceutical

**Methodology Code:** Gas Chromatography
The determination of heat of fusion using differential scanning calorimetry (DSC) for active pharmaceutical ingredients (APIs) and related materials has become more popular due to the availability and the advancement of DSC instruments. Heat of fusion can be used to determine the presence of enantiomers in APIs and provide information about polymorphs. In addition, the purity and the crystallization parameters can be used to further characterize APIs. In the pharmaceutical industry, the identification of polymorphs during the manufacturing process is critical since polymorphs may interfere with the stability of the main component. The heat of fusion of Melting Point Standards (MPSs) that were previously dried and had been gently pulverized was determined using different heating rates. DSC instruments from different manufacturers were used during the evaluation but the results are not intended for instrument comparison. To perform an accurate determination of the heat of fusion of materials, the DSC instruments should be calibrated using high purity standards with well-known predetermined heat of fusion values. DSC data of the heat of fusion determined at several heating rates will be shown and discussed. Currently, there are no United States compendial standards suited for this purpose. Based on the findings, we propose to add heat of fusion values to all of the MPSs. These MPSs could potentially be used for system suitability in compendial applications in harmonized General Chapter <891>. It is proposed to update the label text on all MPSs to include “Heat of fusion by DSC” and update the harmonized General Chapter <891>.
In the modern world of chromatography, numerous options are available to the GC analyst with regard to the method development process. Headspace sampling and injection are commonly chosen techniques for GC analysis; if the sample matrix is non-volatile and the analytes of interest are volatile, the headspace technique in particular can provide consistent peak areas and good sensitivity. In this study, we explored typically overlooked considerations for development of headspace GC methods. Various optimization techniques, including sample preparation and injection styles, inlet parameters, liner selection, and column dimensions were explored in effort to reduce run times and improve peak shapes. The results from this study are then discussed in relation to industry-specific sample matrices from pharmaceutical active ingredients to complex food samples.
Gas Chromatography (GC) sample introduction techniques such as headspace (HS) and automated thermal desorption (ATD) are very useful tools for the investigation of compounds in many matrices and products.

There are several benefits using HS and ATD, as sample and or target introductory techniques, to obtain information about products; for instance, using these technologies, they are significantly less labor intensive providing enhanced productivity; they provide enhanced sensitivity enabling concentration of targets; and they require little to no solvents eliminating analyst exposure to toxic solvents; in addition to being more friendly for the environment.

This presentation will provide a brief overview of theory and function about HS and ATD. However, it will focus on solutions for the chemical, flavor and fragrance, environmental and pharmaceutical industries. For instance, is there an easy, accurate, precise means of measuring the amount of fragrance in a candle?

Methodology from each investigation will be presented to enable others to use these techniques successfully in industry.

The information that can be attained using these technologies and the sample types will be discussed. Also, at times, these technologies are complimentary. Therefore, the presentation will provide recommendations on which technique is best suited for a type of matrix and desired target or information. Information from Flame Ionization Detection (FID) and Mass Spectrometry (MS) Detection will be presented.

Abstract Text:

Gas Chromatography (GC) sample introduction techniques such as headspace (HS) and automated thermal desorption (ATD) are very useful tools for the investigation of compounds in many matrices and products.

There are several benefits using HS and ATD, as sample and or target introductory techniques, to obtain information about products; for instance, using these technologies, they are significantly less labor intensive providing enhanced productivity; they provide enhanced sensitivity enabling concentration of targets; and they require little to no solvents eliminating analyst exposure to toxic solvents; in addition to being more friendly for the environment.

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Two dimensional gas chromatography (GCxGC) has emerged as an effective method of separating complex mixtures. A better understanding of the relative polarities of the primary and secondary column stationary phases would allow for more informed choices in coupling columns, improving separation efficiency. While tabular McReynolds numbers already provide information on a column’s polarity, we have approached presenting polarity graphically on a relative scale to facilitate column pairing. The polarity of six stationary phases commonly used in GCxGC analysis was evaluated. The contributions of the most significant polar interactions: dipole-dipole, proton donating and proton accepting, as well as the magnitude of polar interactions relative to dispersive interactions were determined for each stationary phase. Retention indices were determined isothermally across two temperature ranges, 70-100 C and 150-200 C, using a low boiling and high boiling set of probes, respectively. By comparing the difference in retention indices of each phase against a largely dispersive reference phase, the strength of each polar attribute was determined. The polarity of each phase relative to the other five phases matched between the two probe sets. The contribution of each of the three polar attributes remained constant with increasing temperature for all six phases. Agreeing with past literature, no phase displayed strong proton donating attributes. Polarity relative to dispersive interactions remained constant with increasing temperature across all phases, excluding the biscyanopropyl phase, in which polarity increased with temperature. Results were plotted on selectivity prisms for easy interpretation by GCxGC users. With the exception of the biscyanopropyl phase, the polarity of each phase was determined to be independent of temperature, providing information useful in selecting primary/secondary column temperature.

Keywords: Chromatography, Gas Chromatography
Application Code: Other
Methodology Code: Gas Chromatography
Reversed-phase liquid chromatography (RPLC) using silica-based stationary phases is one of the most widely used separation techniques in chemical analysis. Because of its attractive features, silica continues to be the most popular support material for stationary phase. Surface silanol groups on silica support materials serve as anchors to attach the desired stationary phase. After the best efforts for chemical functionalization, there is a good number of unreacted silanol groups that remain at the silica surface. Such unreacted silanols can provide adsorption sites for nonspecific interactions in the reversed phase separation process. This is troublesome for the separation of basic compounds by RPLC since their interaction with residual silanols results in asymmetrical peaks and irreproducible retention; the use of chromatographic columns with low silanol activity is preferred. It becomes important, therefore, to evaluate RPLC columns for their silanol activity, particularly those that have been introduced most recently (e.g., core-shell). We have evaluated the silanol activity of five commercially available columns reversed phase HPLC columns: one fully porous and four core-shell type (including a hybrid silica). Amitriptyline and dextromethorphan were used to assess silanol activity. Silanol activity was assessed using methanol/water and acetonitrile/water mixtures as the mobile phase, by using the traditional measurement of peak asymmetry. Our results show that the probe compound dextromethorphan has a stronger interaction than amitriptyline towards surface silanols. The interaction becomes more prominent when using acetonitrile instead of methanol in the mobile phase.

Keywords: HPLC, HPLC Columns, Liquid Chromatography
Application Code: Other
Methodology Code: Liquid Chromatography
Total vitamin B12 is extracted in the presence of cyanide from the samples. The extracts are purified and concentrated using immunoaffinity columns and analyzed by ultra-high performance liquid chromatography (UPLC) with tandem mass-spectrometric detection and UPLC with UV detection (AOAC 2014.02). The LC-MS/MS method demonstrated advantage in chromatography of cyanocobalamin in some of the samples in ruling out matrix interference observed in UV based methods. The method accuracy was established by vitamin B12 analysis in a milk based infant/adult nutrition formula NIST standard reference material. The results obtained were within the NIST specifications. The accuracy was further substantiated by evaluation of cyanocobalamin spike recovery by the method in an infant formula/adult nutrition sample. The method provided 94-96% recovery of the spike. The precision of the method in analysis of replicates of the samples on multiple days was found to be satisfactory and ranged between 3.3% to 8.8% as % relative standard deviation. The validated method of vitamin B12 analysis in infant and adult nutrition formulas was found to perform accurately and precisely and exhibited very good specificity.
Abstract Text

Polycyclic aromatic hydrocarbons (PAHs) are compounds containing two or more aromatic rings and are known for their potential carcinogenic and mutagenic properties. They are typically formed during incomplete combustion of organic matter, as industrial byproducts, and in food processing. As such, they are compounds of interest in environmental and food and beverage analysis. Many of these compounds are isomeric, and must be resolved chromatographically to be accurately quantified.

Ionic liquids (IL) are a new class of GC stationary phases that provide unique polar and highly polar selectivity with higher thermal stability compared to traditional siloxane phases with similar selectivity. Traditionally, PAHs have been evaluated by US EPA method 610 using nonpolar stationary phases operated at high temperatures. In this study, we compare the selectivity of the ionic liquid stationary phases to the traditional polysiloxane based phases. We will demonstrate the interesting selectivity of the ionic liquid phases along with introducing a new high temperature phenyl polysiloxane phase for the analysis of PAHs.

Keywords: Capillary GC, Gas Chromatography, PAH, Particle Beam
Application Code: Other
Methodology Code: Gas Chromatography
Polycyclic aromatic hydrocarbons (PAHs) continue to be one of the most studied groups of environmental contaminants because of their high carcinogenic and mutagenic potential. The influence of molecular shape of PAHs in chromatographic separations was first recognized almost 40 years ago for liquid crystalline stationary phases in gas chromatography (GC) and for polymeric C\[i\]C\[sub\]18[/sub]\] stationary phases in reversed-phase liquid chromatography (RPLC). The chromatographic retention behavior of PAHs was correlated to follow several shape selective trends based on the length-to-breadth ([i]L/B[/i]) and thickness ([i]T[/i]) of PAHs. Retention of planar PAH isomers ([i]T[/i] \(\leq 3.90 \text{ Å}\)) was observed to increase with increasing [i]L/B[/i] values and nonplanar PAH isomers ([i]T[/i] \(> 3.90 \text{ Å}\)) eluted earlier than expected based on their [i]L/B[/i] values. Along with PAHs, heterocyclic compounds containing at least one heteroatom such as polycyclic aromatic sulfur heterocycles (PASHs) are largely present in the same environmental samples. The total number of possible isomeric structures for PASH is greatly increased compared with the corresponding PAH because both ring arrangement and position of the heteroatom substitution within the rings give rise to unique isomers. In the present study, column selectivity is examined for GC liquid crystalline and RPLC C\[i\]C\[sub\]18[/sub]\] stationary phases for the separation of PASH isomers. Correlation of the [i]L/B[/i] and [i]T[/i] values for PASHs and their chromatographic retention behavior was investigated.
Dissolved gases in water can originate from a number of sources. It can stem from rock formations, from decomposition of organic material or be produced by microbes. No matter where the dissolved gases come from, they can be hazardous both to the environment and to humans. Don Kampbell and Steve Vandegrift from the Robert S. Kerr EPA lab in Oklahoma developed a standard operating procedure, RSK-175; to test for dissolved light gases through static headspace sampling. However, as these compounds are extremely volatile, a closed sampling system is recommended. Furthermore, RSK-175 is a standard operating procedure and not a formal method; thus, testing procedures for dissolved gases have had a wide range of implementations. The ASTM standard D8028 was proposed to quantitatively determine dissolved light gases in ground waters, waste waters, and drinking waters. This presentation will discuss ASTM Method D8028 and its procedures and requirements.
Analysis of methane and other light hydrocarbons is frequently done as part of predrill background studies to support hydraulic fracturing activities in the various shale plays in the United States. There is no fully validated USEPA method for this type of analysis. ASTM recently released D8028 to address many of the limitations of the previous analytical options. This presentation describes the advantages of this new method and implementation options at the laboratory. At this time there are no commercially available proficiency test samples. Hence the method performance is not documented as well as other common environmental parameters. Internal studies indicate there are opportunities to improve accuracy and reproducibility and provide data more suitable for the oil & gas industry.

Analytical process differences between handling of water samples and gas phase based standards can introduce a low bias. Switching to water phase calibration standards and QC samples can significantly improve the accuracy of the final results. Adding a representative surrogate compound to the sample early in the sample preparation process tracks the integrity of the process. Many fluorocarbon compounds were evaluated to determine which were the best surrogate options and the autosampler adapted to store and deliver the surrogate solution with zero headspace.

Keywords: Environmental/Water, GC, Headspace, Hydrocarbons
Application Code: Environmental
Methodology Code: Gas Chromatography
Environmental Separations

Dual µECD US-EPA Methods Using an Innovative New Gas Chromatographic Platform

US EPA methods such as Contract Laboratory Protocol Pesticides (CLP pesticides) and US-EPA 8081 use a dual column dual detector approach for analysis. One of the columns acts as a primary analysis column while the second column, with different selectivity, is used for confirmation. These methods are widely used for determination of chlorinated pesticides and herbicides in soil and water samples. Unfortunately, many of the legacy chlorinated pesticides are persistent in the environment requiring soil and water testing years or even decades after being banned.

A new concept GC makes installing and maintaining the GC flow path much simpler. No longer will ferrules or periodic trimming at the head of column be necessary. Simpler operation and high sample throughput are key touch points for the new platform.

Traditionally GC results will be shown in comparison with results on the new platform. Tips and guidance on how to best utilize the features of the new platform will be discussed. Our customers have requested simpler to operate gas chromatographs and our latest innovative designs answer their request with gusto.

Keywords: Environmental Analysis, Gas Chromatography, GC Columns, High Throughput Chemical Analysis
Application Code: Other
Methodology Code: Gas Chromatography
Environmental Separations
Linear and Equimolar Response of Hydrocarbons, Oxygenates and Highly Functionalized Organic Compounds Over 7 Orders of Magnitude with Reaction-FID

The variable response of gas chromatography detectors to different compounds can introduce error into the quantitative analysis of those compounds and leads to the need for careful calibrations of the detector for each compound. Here, we show how the sequential oxidation and reduction of organic compounds to methane can lead to a universal equimolar response in the flame ionization detector (FID). The response is linear over nearly 7 orders of magnitude with a high sensitivity that is essentially independent of molecule type from 0.1 ppm to pure substances. The technology is simple and stable over thousands of injections with RSD values similar to those of the FID. The detector has an unparalleled uniform response to carbon-containing compounds including CO, CO2, organic acids, aldehydes, alcohols, ethers, ketones, hydrocarbons, halides, and more. This uniform response allows for enhanced quantification with a reduction of calibrations, increased accuracy and diagnostic capabilities for the GC.

Keywords: Calibration, Gas Chromatography, GC Detectors, Volatile Organic Compounds
Application Code: General Interest
Methodology Code: Gas Chromatography
Ingestion of water or aquatic food products contaminated with cyanotoxins produced by cyanobacteria (blue-green algae) may cause severe illness or death in humans and animals. We are developing a method for the rapid identification and quantification of toxin-producing cyanobacteria in mixed-species blooms impacting New York State waters using Liquid Chromatography-Tandem Mass Spectrometry. The goal is to establish a more comprehensive and cost-effective technique than ELISA, which is currently in use for the analysis of cyanotoxins. Cyanotoxins studied in this research project include groups of anatoxins, cylindrospermopsins, microcystins, and nodularin. The method will be validated in fresh water cyanobacterial extracts and aquatic animals living in New York State. Results from this study will not only provide valuable data regarding the presence and levels of cyanotoxins in fresh water in New York State, but will also be important in ensuring the capability and capacity to respond to emergencies involving widespread contamination of cyanotoxins in aquatic environments and food products.

Keywords: Environmental/Water, Extraction, Food Safety, Liquid Chromatography/Mass Spectroscopy
Application Code: Other
Methodology Code: Liquid Chromatography/Mass Spectrometry
Environmental Separations

Optimizing the Analysis of Semi-Volatiles by EPA Method 8270

The results of this study show how the Thermo Scientific ISQ™ Series Single Quadrupole GC-MS system can meet United States Environmental Protection Agency (U.S. EPA) 8270D Method requirements. Thanks to the extended dynamic range detection system, the method range was 0.2-200ppm using the same column. The new Thermo Scientific™ Instant Connect Helium Saver Module was assessed in this study to show that significant financial costs savings can be realized throughout the lifetime of the GC-MS instrument without compromising the instrument’s performance.

Keywords: Environmental Analysis, Gas Chromatography/Mass Spectrometry, Mass Spectrometry, Water

Application Code: Environmental

Methodology Code: Gas Chromatography/Mass Spectrometry
Chromatographic separations take place in a competitive environment in which a limited number of adsorption sites are competed for by numerous molecules with differing properties. Most mechanistic studies of chromatography, however, have focused on model systems with a single adsorbate. Here we use single molecule spectroscopy and a novel competitive isocratic chromatographic operating regime to study a multicomponent, competitive chromatographic system. Fluorescently-labeled β-lactalbumin proteins are imaged at super-resolution levels adsorbing to spermine anion-exchange ligands as a function of different concentrations of a competitor, unlabeled insulin. We observe that the adsorption kinetics of β-lactalbumin at single ligands are unchanged in the presence of insulin. Instead, we find that the competitor reduces the number of binding events among the available spermine ligands. Super-resolution imaging reveals that the reduction in events is caused by the competitor blocking ligands throughout the observed measurement time, while other spermine ligands remain accessible to the analyte. Isocratic chromatographic retention using an approach where competitor concentration is held constant is measured and is directly compared to the single molecule results using the stochastic theory of chromatography. Similar trends in peak asymmetry are observed between the single molecule and column elution curves, but deviations are also seen in retention times caused by differences in ligand density, geometry, and mass transport. Overall, we have expanded mechanistic single molecule spectroscopic studies of chromatography to multicomponent samples and comparisons to functional ion-exchange columns to show that competitors block certain ligands from analytes without changing others, likely due to a slow desorption time of the competitor on the time scale of the measurements.

Keywords: Fluorescence, Imaging, Ion Exchange, Liquid Chromatography
Application Code: Pharmaceutical
Methodology Code: Fluorescence/Luminescence
Spatially Resolved Photoluminescence Reveals Spectral Heterogeneities in Organic-Inorganic Lead Halide Perovskite Microcrystals

Organic-Inorganic lead halide perovskites (with general formula AMX$_3$ [A: CH$_3$NH$_3$+, HC(NH$_2$)$_2$+; M: Pb$_2$+; X=Cl-, Br-, I-]) have attracted interest due to their exceptionally high photoluminescence quantum yields, charge carrier lifetimes, carrier diffusion lengths, and easy solution processability at low temperatures[1-5].

Despite these attractive properties, non-uniformities arising from common deposition processes would be expected to play a significant role in determining properties of photovoltaic, light-emitting diode, and lasing devices fabricated from solution-processed perovskite materials. Our spatially resolved photoluminescence measurements reveal significant spectral differences between individual locations on solution-deposited polycrystalline methylammonium lead bromide (CH$_3$NH$_3$PbBr$_3$) thin films. Excitation of the samples with a spatially localized, focused laser beam allows us to compare the photoluminescence intensity and peak wavelength at different locations within the film with respect to the emission at the direct excitation spot. Particularly, we find that the behavior of spectral emission within the bulk of a constituent single crystalline grain is markedly different from that at its boundaries. From our results, we posit these effects to be results of a combination of wave-guided out coupling, self-absorption of photoluminescence, charge carrier diffusion and intraband energy relaxation. Our results pave the way for a better understanding of the emission characteristics of perovskite films, and assign specific contributory mechanisms to the observed spectra.

References:


Keywords: Laser, Luminescence, Material Science, Microscopy

Application Code: Material Science

Methodology Code: Fluorescence/Luminescence
In a conventional fluorimeter, optimization of sensitivity in relation to sample geometry is achieved by optimizing the overlap of the observation field of the detector and the field of excitation light in the sample compartment. At this level of sensitivity, often, certain environmental fluorophores present at very low concentration can’t be estimated without prior pre separation. This work proposes the use of (a) larger observation volume by suitable sample geometry and detector configuration, (b) use of reflective coating on the wall of the container to increase light intensity within the observation volume. An intensity enhancement up to one order of magnitude is observed. Conventionally fluorescence signature is captured at right angle geometry to the excitation radiation; but we verified an oblique incidence of excitation radiation and collection of fluorescence from top of sample compartment. This practice minimizes the contamination of excitation light with fluorescence light. A correction factor is developed to counter the wavelength dependence of the reflective surface. Such a practice essentially enables the analyst to adapt this method for both qualitative and quantitative estimations. The sample compartment is designed to suit both on-line and laboratory based experiments. This method is essentially a cost-effective and simple approach towards sensitive fluorescence detection.

Reference:

Keywords: Environmental/Waste/Sludge, Environmental/Water, Fiber Optics
Application Code: Environmental
Methodology Code: Fluorescence/Luminescence
Our understanding of biology can be greatly improved if we could analyze more co-localized biomarkers in situ than currently possible. Spectral overlap between fluorophores’ broad peaks creates spectral cross-talk between detectors. Spectral cross-talk makes accurate signal differentiation from co-localized emitters difficult. Currently, less than 30% spectral overlap is considered acceptable to mitigate cross-talk. The acceptable level of spectral cross-talk needs to be increased to increase the number of co-localized emitters simultaneously analyzed. To achieve this goal, we present a co-localized excitation-emission resolution (CLEER) technique. CLEER uses a prediction methodology and fluorescence analysis approach to reduce cross-talk from co-localized emitters. The prediction method seeks to identify dye specific excitation-emission coordinates (DySEEC) for each emitter in a given mixture. The fluorescence analysis approach will facilitate selective excitation and emission collection at each dye’s DySEEC. The fluorescence technique uses a tunable ultrafast laser for selective two-photon excitation of each dye. Two thin-film linear variable chromatic filters create a bandpass filter that is tunable in both the center wavelength and the bandpass. The tunable emission filter enables selective emission collection from each dye. The prediction method and fluorescence system are automated with matlab. Cross-talk from dyes in cell lysate using DySEECs will be compared to conventional spectral resolution techniques. The ability of CLEER to increase the number of dyes that can be analyzed will be discussed.

Keywords: Bioanalytical, Fluorescence, Method Development, Spectroscopy
Application Code: Bioanalytical
Methodology Code: Fluorescence/Luminescence
Biochemists are investigating detection methods for biological substances such as protein, and neurotransmitters. In this study, we have designed and synthesized two fluorescent molecular probes based on cyanopyranyl fluorophores; i) fluorescent reagent using FRET between dansyl fluorophore as energy donor and cyanopyranyl group as energy acceptor for the detection of glycoconjugates (Probe 1) in combination with magnetic beads, ii) fluorescent molecule for the highly sensitive and selective detection of dopamines (Probe 2).

In order to detect lectin-saccharide interactions using a novel FRET pair (Probe 1), the dansyl fluorophore and lectins were immobilized on the surface of magnetic particles, and the detections of various saccharide containing cyanopyranyl moiety were successfully performed in highly sensitive (limit of detection was 0.1 nM) way. Moreover, this analytical system recognized the structural difference between glycoprotein and non-glycosylated proteins, and performed a highly sensitive detection of glycoconjugates.

Probe 2 was constructed using the cyanopyranyl group as the fluorophore and Fe(II) complex both as the ligand exchange site. In contrast to the weak fluorescence emission of Probe 2 in the absence of dopamine, a much stronger fluorescence emission was observed following the addition of dopamine owing to the release of Fe(II) from Probe 2. The reaction of Probe 2 with dopamine was not affected by the presence of foreign substances, thereby allowing for the highly selective detection of dopamine.

These results proved that above two fluorescent probes could be useful for a convenient and highly sensitive detections of biological substances in biochemical and medical fields.

Keywords: Biosensors, Fluorescence, Molecular Spectroscopy
Application Code: Bioanalytical
Methodology Code: Fluorescence/Luminescence
Fluorescence and Luminescence Advances

Metal Enhanced Fluorescence on Gold Nanostars: Role of Nanostar Plasmon Band and Fluorophore Spectrum Overlap

Nanostars have gained popularity as contrast agents and to enhance Raman signals because the enhanced electric fields are localized on the individual nanostar. The reason for this localization is due to many sharp spikes on each nanostar. Metal enhanced fluorescence (MEF) from noble metal colloidal solutions depends on the spectral overlap between the nanostar’s plasmon band and the fluorophore’s excitation and emission spectrum. Fluorophores that are within 10’s of nanometers of a nanomaterial can have the fluorescence emission enhanced by near-field and far-field enhancement. In the near-field domain, the fluorophore’s absorption/excitation spectrum needs to overlap with the plasmon band. For the far field domain, the fluorophore’s emission spectrum needs to overlap with the plasmon band. We will discuss the near-field and far-field effects for gold nanostar MEF. Fluorescence from dyes with different excitation and emission spectra were evaluated as a function of their spectral overlap with the nanostar plasmon band. The relationship between observed fluorescence intensity and the spectral overlap between gold nanostar plasmons and spectra of each fluorophore will be discussed.

Keywords: Bioanalytical, Fluorescence, Molecular Spectroscopy, Nanotechnology
Application Code: Bioanalytical
Methodology Code: Fluorescence/Luminescence
The abuse of emerging synthetic drugs in this country as an alternative to more historic illegal forms is a growing problem. To prevent this abuse, many states have passed laws banning these drugs, and the DEA has begun scheduling and temporarily scheduling these drugs. Due to this problem and the lack of research being done, crime laboratories are having difficulty identifying and analyzing these drugs efficiently. Therefore, a single analytical method was developed using GC-MS/FID to identify and quantify different classes of synthetic drugs so that crime laboratories can analyze these drugs quickly and increase their productivity. GC-MS was used to identify the synthetic drugs, and a library of retention indices was created from reference standards using an aliphatic hydrocarbon standard mix as a better form of identification rather than relying on mass spectral libraries alone. GC-FID was used to quantify the drugs due to Leffler et al.’s documented reactivity of some of the synthetic drugs when using MS. This method successfully separated six classes of synthetic drugs and was used to identify and quantify synthetic drugs in street samples. However, it was determined that derivatization of the 2Cs was needed for optimal separation performance due to the reactivity of the compounds. To date, only two of the synthetic cathinones posed a problem of co-elution, and thus could only be semi-quantified. Method precision (as %RSD) was relatively low for all the compounds with the synthetic cannabinoids showing the best precision and the synthetic cathinone, methylone, showing the poorest. Utilizing retention indices has been demonstrated to be a clearly better method for identification than reliance on mass spectral data alone, especially for compounds that are isomers and have similar mass spectra. This method will provide a more systematic method for the analysis of synthetic drugs, allowing crime laboratories to keep up with this growing problem.

Abstract Text

Keywords:
- Drugs
- Forensic Chemistry
- Gas Chromatography

Application Code:
- Other

Methodology Code:
- Gas Chromatography
Cannabis is one of the world’s most frequently used drugs of abuse and with recent legalisation across multiple states of the USA, the need for robust testing of cannabis products is on the rise. However, this can be a challenging prospect due to the sample and matrix complexity.

In this study, we focus on the use of comprehensive two-dimensional gas chromatography coupled with time-of-flight mass spectrometry (GCxGC-TOF MS) for improved separation capacity in the analysis of these complex samples of plant origin.

We will also examine a number of so-called ‘legal highs’, which have gained notoriety for their ability to circumvent current legal controls, due to their novel structures. The constant emergence of compounds with new structures has made it difficult for researchers to keep pace and conduct full investigations into their composition.

To aid the characterization of these novel compounds, an innovative ion source design is employed, to generate both hard and soft electron ionisation (EI) spectra simultaneously. This technique, known as Tandem Ionisation, is proven to aid structural elucidation of isomers and enhance confidence in identification of compounds where there are no reference spectra available.

Keywords: Drugs, Forensic Chemistry, GC-MS
Application Code: Consumer Products
Methodology Code: Separation Sciences
Abstract Text

Originally used to describe a class of compounds specific to the plant Cannabis sativa, the term cannabinoid now encompasses all molecular species that are capable of binding to the cannabinoid receptor within humans. This includes the naturally occurring phytocannabinoids, endogenous endocannabinoids, and synthetic cannabinoids. With the constant emergence of new synthetic analogues and continual changes in legislature, combined with the vast number of analytes to be monitored, new challenges have arose for drug testing laboratories. Therefore, the development of reliable, simplified, and rapid sample preparation techniques that can be coupled with specific LC-MS based analysis is essential. Biocompatible solid phase micro-extraction (BioSPME) is a technique that allows for the selective extraction of analytes of interest without significant co-extraction of matrix interferences. The method is an equilibrium based approach, with only the free fraction of analyte being collected from the sample. Therefore, sample pre-treatment such as protein precipitation or cell lysing is typically not necessary. A variety of cannabinoids were successfully extracted using the BioSPME method. Absolute recoveries were above 80% with relative standard deviations below 6%, on average, identifying BioSPME as an accurate and precise methodology. BioSPME also resulted in minimal matrix effects and reduced total MS background. Thus, allowing for lower detection limits and reduced frequency of instrument cleaning and maintenance compared to more traditional approaches, such as protein precipitation or “dilute and shoot”. In addition, the pipette tip format is high throughput amenable, allowing for a more rapid sample preparation alternative.
A direct deposition infrared spectrometer coupled with a gas chromatograph (GC-IRD) provides a reasonable alternative/complimentary technique to traditional instrumentation. Forensic exhibits come in a multitude of forms, including residues, liquids, powders and other matrices routinely observed by the forensic drug chemist. The GC-IRD has demonstrated the ability to differentiate positional isomers of designer drugs of abuse such as the isomers of 3,4-methylenedioxymethamphetamine (MDMA), synthetic cannabinoids, NBOMe and other phenethylamine type compounds. The instrument can also be utilized as a second technique/second sampling resulting in more robust data. The GC-IRD utilizing solid phase deposition is a capable instrument in the analysis of compounds commonly encountered in the forensic drug chemistry laboratory. Discussion will include presentation of data as well as QA/QC and operational suggestions.
The olfactory tubercle (OT), as a component of the ventral striatum, serves as an important multisensory integration center for reward-related processes in the brain. Recent studies show that dense dopaminergic innervation from the ventral tegmental area (VTA) into the OT may play a role in disorders such as psychostimulant addiction and disorders of motivation, increasing interest in this brain region. However, due to its anatomical inaccessibility, relative small size, and proximity to other dopamine-rich structures, neurochemical assessments using conventional methods cannot be readily employed. Little is known about DA regulation in the subregions of the OT, the contribution of other neurotransmitters such as γ-Aminobutyric acid (GABA) in modulating DA, and its impact on behavior. Recently, we investigated DA regulation in the OT of urethane-anesthetized rats using in vivo fast-scan voltammetry (FSCV) coupled with carbon-fiber microelectrodes, following optical stimulation of the VTA expressing channelrhodopsin-2 (ChR2), a non-native light sensitive cation channel, whose expression was driven by a generalized non-restricted promotor. However, the VTA is a highly heterogeneous brain region containing many different types of neurons including DA and GABA. Therefore the use of a generalized non-restricted promotor results in ubiquitous expression of ChR2 in all neurons in the VTA. Here we will demonstrate to target ChR2 expression to DA neurons in the VTA and are parsing the role of this and other neurotransmitters in OT subregions using FSCV and a recently developed viral system.

Keywords: Electrochemistry, Neurochemistry, Optogenetics
Application Code: Neurochemistry
Methodology Code: Electrochemistry
Dopamine (DA) circuitry in the dorsal and ventral striatum are broadly implicated in movement and reward. DAergic cell bodies in the ventral tegmental area (VTA) and substantia nigra projecting to the striatum terminate largely in the nucleus accumbens (NAc) and dorsal striatum (DS), respectively. However, the mesolimbic and striatonigral pathways, thought to be largely functionally distinct, may overlap more than previously thought. A recent opto-fMRI study demonstrated that optogenetic stimulation of dopamine neurons in the VTA of TH::Cre rats produces larger increases in blood oxygenation-level-dependent (BOLD) signals in the DS compared to NAc (Lohani et al., 2016). Guided by this observation, we investigated dopamine dynamics in DS and NAc during activation of VTA DA neurons. We selectively stimulated DA neurons in the VTA of Th::Cre rats expressing channelrhodopsin-2 (ChR2) and monitored DA overflow in the DS and NAc, by both sampling DV coordinates in the dorsal and ventral striatum, and simultaneous monitoring of the DS and NAc. We used carbon fiber microelectrodes to detect DA using fast scan cyclic voltammetry. This measure of DA dynamics in the striatum (n=3) so far suggests that DA spillover in the DS may be larger than that in the NAc and exhibits profoundly different kinetic profiles. Additionally, we compare electrical stimulation of the VTA to optogenetic stimulation. Currently, we are employing the restricted diffusion model for DA dynamics to describe the kinetics of the responses, and to determine rate constants for DA release, transport, and uptake in different locations throughout the striatum.

ER and SL are co-first authors.


Keywords: Electrochemistry, Neurochemistry, Optogenetics, Voltammetry

Application Code: Neurochemistry

Methodology Code: Electrochemistry
Nicotinic acetylcholine receptors (nAchRs) are ligand-gated ion channels that respond to acetylcholine and nicotine in insect central nervous system. The receptors exist as pentamers of different combinations of subunits (1 – 7 and 1 – 3 in Drosophila). It has been suggested that dopaminergic pathways in Drosophila modulate the response to nicotine as a natural insecticide. Neonicotinoid insecticides are synthetic analogs of nicotine that have longer field stability and lower mammalian toxicity. We have studied nAchR mediated dopamine release in Drosophila larval ventral nerve cord. nAchR agonist is pressure ejected and resulting response measured with fast scan cyclic voltammetry (FSCV) at carbon fiber microelectrode with the triangular dopamine waveform. Flies that are fed dopamine synthesis inhibitor, 3 iodotyrosine, have lower stimulated release suggesting the response in due to dopamine. Acetylcholine stimulated release is stable upon repeated stimulation while nicotine stimulated release lasts much longer and keeps increasing upon repeated stimulation. Neonicotinoids also stimulated release, which had similar characteristics as nicotine stimulated release. In recent years, insects resistant to neonicotinoids have been identified, and mutations in regions of D1, D2 and D3 nAchR subunits generated Drosophila strains, which were highly resistant to neonicotinoids nitenpyram and imidacloprid. Neonicotinoid-stimulated release is significantly lower in the D1, D2 and D3 nAchR subunit mutants suggesting that the insecticide acts on the mutated regions of the subunit. We have identified a novel preparation to study the direct effect of nAchR subunit mutations on neonicotinoid mediated response. This will help understand the subunit specificity of different neonicotinoids and identify nAchR subunit mutations that can cause insecticide resistance.
Serotonin plays important roles in brain physiology and is linked to a number of affective disorders, including depression. A surprisingly large number of neuropharmacological agents that specifically target serotonin have low efficacy rates. This problem arises primarily from our lack of understanding of the principle mechanisms governing extracellular serotonin and how pharmacology modulates serotonin levels. To better understand the fundamental mechanisms that control extracellular serotonin, we utilize fast scan cyclic voltammetry and fast scan controlled adsorption voltammetry to measure stimulation evoked and ambient serotonin in three different brain regions. We find that both evoked and ambient serotonin profiles are different between regions and hypothesize the differences to arise from physiology local to the voltammetric probe. We employ 2-photon microscopy and mathematical modeling to test and verify the hypothesis brought forth from voltammetric data. We thus present our measurements as uniquely powerful for deciphering the synaptic mechanisms that regulate serotonin in health and disease.

**Keywords:** Electrochemistry, Neurochemistry, Pharmaceutical

**Application Code:** Neurochemistry

**Methodology Code:** Electrochemistry
Measuring Dopamine and Serotonin In Vivo

Coregulation of Serotonin and Histamine in the Context of Neurodegeneration

Neurodegeneration underlies incurable and difficult to treat disorders such as Parkinson’s disease. Not knowing the fundamental chemical etiology of these illnesses make them challenging to prevent, diagnose and cure. To establish the underlying chemistry of these disorders, it is critical to understand the dynamic [i]in vivo[/i] behavior of neurotransmitters during disease progression. While there is much emphasis on dopamine chemistry during neurodegeneration, serotonin and histamine remain relatively understudied but may also play key roles. In this work, we develop a fast scan cyclic voltammetry method to simultaneously and selectively monitor serotonin and histamine in real time [i]in vivo[/i]. Histamine release is evoked in the premammillary nucleus of the mouse brain via electrical stimulation of the medial forebrain bundle. We illustrate that histamine release causes rapid inhibition of serotonin via H3 receptors. Furthermore, mathematical and pharmacological experiments imply an active uptake mechanism for histamine. Finally, we apply these measurements in various mouse models of neurodegeneration. Our method offers the opportunity to study the roles of serotonin and histamine, two important neurotransmitters, in neurodegeneration processes.

Keywords: Bioanalytical, Electrochemistry, Microelectrode, Neurochemistry

Application Code: Neurochemistry

Methodology Code: Electrochemistry
The recent rise in the number of parents choosing to not vaccinate their children is leading to an increased chance of a potential epidemic. A recent resurgence of preventable diseases such as measles and whooping cough further validate this concern. One of the primary arguments of anti-vaccine advocates is that the mercury containing antiseptic, thimerosal, causes autism. To date, there have been conflicting studies regarding the severity of the health hazard thimerosal poses. In light of this, we endeavored to investigate the neurotoxic effect at a very basic level. We utilized fast scan cyclic voltammetry (FSCV) to measure evoked serotonin levels before and after administering acute and chronic thimerosal doses to determine the effects on neurotransmitters. We conclude that while thimerosal does cause disturbances to the serotonergic system at high doses, there are no observable differences at concentrations reflective of those used in vaccines.

**Keywords:** Mercury, Neurochemistry, Toxicology, Voltammetry

**Application Code:** Neurochemistry

**Methodology Code:** Electrochemistry
Abstract

Spatial electrochemical monitoring at present has been effectively used in the brain and on single cells to mainly monitor heterogeneity of transmitter release. In comparison, only a handful of research efforts have been dedicated to the intestinal tract, where numerous single point monitoring studies in the intestinal tract have been conducted. Serotonin is released from enterochromaffin cells which sparsely populate the mucosal lining of the intestinal tract. The intestine is a large organ and studies have failed to fully understand how the release profile of serotonin alters in the entire colon. Therefore this study aims at developing and characterising an electrochemical array capable of monitoring release of serotonin from the entire colon of a murine in order to understand how patterns of release varied. We created a 3D printed mould pattern that could be utilised to house 6 carbon nanotube composite electrodes. These devices were created so that the tissue would sit directly above the electrodes at a fixed distance. The developed array was assessed reproducibly, stability and fouling. The array was assessed by monitoring levels of serotonin from the whole colon from a murine in the presence of pharmacological agents. Arrays were characterised in potassium ferricyanide and serotonin. Fouling measurements were carried out to see if any attenuation of the potassium ferricyanide response was observed pre- and post- biological measurements where a 10 % reduction was observed. Differential pulse voltammetry (DPV) tests showed signalling corresponding to the release of serotonin and melatonin along the length of the colon. Addition of prucalopride, a 5-HT4 agonist showed a significant reduction in the current. This novel composite electrode array provides pharmacological and physiological insight into signalling patterns in the intestinal tract and can provide useful knowledge in areas for drug targeting.

Keywords: Biological Samples, Electrochemistry, Electrodes, Neurochemistry

Application Code: Neurochemistry
Methodology Code: Electrochemistry
A great deal of progress has been made in mapping the genetic abnormalities associated with diseases such as cancer and diabetes. Unfortunately, the same is not true for the environmental effects associated with these chronic diseases. Untargeted profiling methods are critical for effectual investigation of this relationship in hope of preventing, treating or reversing diseases. Metabolomics is a particularly useful tool for this work due to its proximity to system phenotype and its relatively quick insight into environmental perturbations from drugs, food, smoke and pollutants. Challenges associated with metabolomics include the enormous number of chemically diverse metabolites present in a wide range of concentrations. The main bottleneck in metabolomics continues to be the structural characterization of compounds in complex biological matrices.

The objective in this study was to develop new methodology for routine and effective analysis of biological samples. Rapid and comprehensive profiling of compounds in these samples was accomplished using a novel, high performance benchtop time-of-flight (TOFMS) instrument. Confirmation and discovery modes of analysis were carried out by incorporating enhanced chromatographic resolution and increased mass spectral resolving power to sample measurements using high resolution TOFMS instrumentation. The application of this multiplatform workflow allowed for rapid but more importantly, confident annotation of metabolites in human plasma. Our methodology also included untargeted data acquisition using different ionization methods to achieve higher numbers of confidently identified metabolites. In addition, software tools were used to quickly reinterrogate rich data sets in a targeted manner to improve sample throughput after compounds of interest were identified using comprehensive Peak Find processing.

**Keywords:** Gas Chromatography/Mass Spectrometry, Metabolomics, Metabonomics

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

**Methodology Code:** Gas Chromatography/Mass Spectrometry
Chiari type I malformation is a condition characterized by herniation of the cerebellar tonsils through the foramen magnum. Patients experience disabling neurological symptoms such as chronic pain, disturbances in balance, and numbness. Magnetic resonance imaging (MRI) is currently used for diagnosis; however, the extent of a herniation is not always related to severity of symptoms complicating the diagnosis and treatment of this disorder. We have performed a global metabolomic analysis of cerebrospinal fluid (CSF) and blood from Chiari patients in order to identify biochemical pathways associated with neurological symptoms. CSF and serum samples were collected from Chiari subjects as well as patients diagnosed with hydrocephalus who served as non-Chiari controls. Metabolites were extracted with methanol and analyzed by using hydrophilic interaction chromatography-mass spectrometry (HILIC-MS) with a triple quadrupole time of flight instrument. We find alterations in monoamine neurotransmitters, including 3-O-methyldopa, dopamine, and serotonin, in Chiari CSF compared to the CSF isolated from individuals diagnosed with hydrocephalus. Fewer significant metabolite alterations are seen in blood although decreases in serotonin are detected in both biofluids. Our results suggest that Chiari patients exhibit a unique pattern of metabolic disturbances in the CSF that result from the herniation. Therefore, the use of metabolite biomarkers in conjunction with MRI measurements of herniation holds promise for the diagnosis of Chiari patients.

This work was supported by Conquer Chiari Center and AB Sciex.
Opioids are one of the frontline drugs administered for the treatment of chronic pain symptoms. Unfortunately, the prolonged use of opioids often results in a paradoxical situation called opioid-induced hyperalgesia (OIH), where the patient becomes more sensitive to pain stimuli. Though the molecular mechanisms of OIH are not well characterized, the neuropeptides which act as cell-to-cell signaling molecules between the different brain regions of the pain signaling pathway are believed to play a major role. To investigate the identity of the peptides correlating with OIH, we performed a label-free mass spectrometry based relative quantitation between OIH-induced and control group of mice. This method, facilitated via Skyline, open-source Windows client application aiding MS1 quantitation, has helped us take advantage of the wider dynamic range of analyte concentrations, better coverage and simpler sample processing of label-free techniques compared to quantitation methods using isotopic labels. With this approach, a total of 220, 246 and 409 peptides were quantified in the regions of dorsal horn, trigeminal nucleus and trigeminal ganglion respectively. Of all the quantified peptides, 28 were found to be significantly up or down-regulated in the studied regions and 17 of these 28 were derived from prohormones, including Calcitonin-gene-related peptide, Secretogranin-2, Neurotensin, pro-Enkephalin-A and pro-Neuropeptide-Y which have been previously shown to be involved in pain related processes. The peptide candidates identified in this study will help elucidate the mechanistic processes leading to OIH.
Metabolomic Profiling of Food Diets Using Ion Chromatography with High Resolution Mass Spectrometry

Analysis of small polar metabolites is critical to understanding many of the metabolic disturbances as a result of disease, lifestyle, and diet. In this experimental design, UC Davis Center of Metabolomics generated three food samples representing three different diets: low animal protein (Davis), high fish and vegetables (California), and high beef, high sugar, and high fat (USA). Recently it has been shown that ion chromatography (IC) when combined with HRAM MS can provide superior separations and sensitivity for polar ionic species as compared to other LC methods. These results have been demonstrated using a capillary IC and replicated using a higher throughput IC system.

Over 1028 compounds were detected in the food samples, of which about 180 small polar compounds were assigned using IC-HRAM via software Compound Discoverer v2.0. Compound Discoverer utilizes mzCloud and ChemSpider to identify compounds, based on MS/MS and accurate mass, respectively. Succinate, as an example, was confirms by comparing the MS/MS spectra with mzCloud library, and we displayed the results found in each sample and diet.

Many of the small polar compounds found by IC were central carbon metabolism compounds, i.e., TCA metabolites. As an illustration of IC-HRAM analysis coverage, these compounds and their differences were summarized in the cycle. Monophosphate and diphosphate sugars and nucleotides, such as AMP were typically difficult to resolve by HILIC and RP chromatography methods, they were also well resolved and easily detected by IC-HRAM. In this study these samples were analyzed using IC-Orbitrap MS and processed using Compound Discoverer 2.0.

Keywords: Ion Chromatography, Liquid Chromatography/Mass Spectroscopy, Mass Spectrometry, Metabolomic

Application Code: Genomics, Proteomics and Other ‘Omics

Methodology Code: Liquid Chromatography/Mass Spectrometry
Capillary Microsampling CE-ESI-MS Enables Analysis of Metabolites in Single Embryonic Cells of the Developing Frog (Xenopus) Embryo

Characterization of the metabolome in single-cells provides a better understanding of processes taking place during cell differentiation. Using single-cell mass spectrometry (MS), we have recently detected diverse types of metabolites in single embryonic cells that have been dissected from the embryo. Here, we advance single-cell MS workflow to enable the direct, in situ analysis of metabolites in multiple cells in the developing embryo. First, we adapted capillary microsampling to our custom-build single-cell MS instrument, and validated the technology. Next, we used this technology to explore metabolic cell heterogeneity among dorsal and ventral cells in the 16-cell Xenopus laevis frog embryo that give rise to different tissue types. Using capillary microsampling MS, we were able to aspirate an ~1–10 nL portion of each identified cell’s cytoplasm and then micro-extract endogenous metabolites from these samples. Metabolite extracts were analyzed using a custom-built volume-limited capillary electrophoresis electrospray ionization platform in conjunction with a time-of-flight mass spectrometer. Surprisingly, capillary microsampling revealed a comparable number of molecular features to whole-cell dissection despite collecting ~1–10% material from single cells. Of a total of ~250 molecular features detected between cells, we identified 55 as small molecules (metabolites) based on accurate mass measurements, tandem MS, and migration time comparison to standards. Furthermore, using multivariate data analysis strategies (PCA, HCA, PLSDA), we found metabolite gradients across the cell types. The technology presented in this work raises a potential to understand how metabolic changes contribute to cell differentiation during development of the vertebrate embryo or other animal models.

Keywords: Capillary Electrophoresis, Mass Spectrometry, Metabolomics, Metabonomics
Application Code: Genomics, Proteomics and Other ‘Omics
Methodology Code: Mass Spectrometry
Metabolomics, Proteomics, and Lipidomics

[i]Pseudomonas Aeruginosa[/i] Proteomics for Models of Multispecies Biofilms

[i]Pseudomonas aeruginosa[/i] is an opportunistic gram negative bacterium that is commonly found with other bacteria in chronic wounds. Understanding the interspecies interaction in such multispecies biofilms is essential for devising effective therapies. The expression of transport and other membrane proteins can be used to follow metabolic pathways and elucidate mechanistic behavior of multispecies biofilms. We apply cellular membrane subfractionation of [i]P. aeruginosa[/i] isolate from a chronic wound patient, followed by shotgun liquid chromatography tandem mass spectrometry-based proteomics. Preliminary results from this extraction and proteomic analysis identified over 300 membrane proteins. Included in the transport proteins so identified are the tripartite multidrug transporter mexAB-oprM efflux responsible for multidrug resistance, siderophore receptor involved in inorganic ion transport, ABC transporters, and transporters of nucleotides, lipids, carbohydrates and amino acids. We will discuss how such proteomic data can be used to inform computational models of multispecies biofilms.

Keywords:
- Biomedical, Isolation/Purification, Liquid Chromatography/Mass Spectroscopy, Proteomics

Application Code:
- Genomics, Proteomics and Other 'Omics

Methodology Code:
- Liquid Chromatography/Mass Spectrometry
Renal cell carcinoma (RCC) is among the 10 most common cancers. More than 50% of the patients exhibit locally advanced or metastatic RCC at the time of diagnosis and have a poor prognosis (5-year survival rate <2%). RCC consists of several histological subtypes with diverse molecular alterations. Clear cell RCC (ccRCC) is the most common (75%), lethal subtype. Current research has shown that several metabolic alterations are associated with ccRCC tumor progression and metastasis. However, more studies are needed to find robust biomarkers for early diagnosis and prognosis.

In this pilot study, untargeted lipidomic profiling of age-matched serum samples from 5 patients with advanced ccRCC (stage IV) and 5 healthy individuals was performed using ultraperformance liquid chromatography coupled to quadrupole-time-of-flight mass spectrometry. High resolution mass spectra were acquired in negative ionization mode across the range of m/z 50–1200. Metabolites were extracted from the serum samples using isopropanol. Metabolic features (Rt, m/z pairs) were obtained via Progenesis QI software and analyzed using a cross-validated orthogonal projection to latent structures-discriminant analysis model. This supervised classification model distinguished sample classes with high accuracy (R²Y=0.9985 and Q²Y=0.9956). Discriminant metabolic features suggest alterations of glycerophospholipids and cholesterol metabolism. Our current work involves the retrospective analysis of a larger cohort (n=240) that includes serum samples from ccRCC patients with stages I, II, III, and IV before and after surgery and healthy individuals to shed light into the altered metabolic pathways involved in tumor progression and to discover biomarkers useful for early diagnosis, prognosis, and follow-up care.

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.

**Keywords:** Data Analysis, Lipids, Liquid Chromatography/Mass Spectroscopy, Metabolomics

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

**Methodology Code:** Liquid Chromatography/Mass Spectrometry
Higher chromatographic resolution is needed for top-down proteomic analysis of histones, which are involved in epigenetic control of a wide variety of cellular processes through their multiple post-translational modifications. Histones are strongly cationic, which makes them challenging to separate with reversed-phase liquid chromatography coupled to mass spectrometry (RPLC-MS), where trifluoroacetic acid cannot be used. RPLC with and without MS is performed on a histone sample using trifluoroacetic acid vs. difluoroacetic acid and a 20-minute gradient. Columns with C18 surfaces are compared for different types of particle morphologies: 1) fully porous particles of 5 [micro]m in diameter, 2) superficially porous particles of 3 [micro]m in diameter with a shell of 0.2 [micro]m, and 3) nonporous particles of varying diameter. The results show the roles of particle morphology, acidic modifier and bonded phase in resolving intact histones in RPLC-MS.

Keywords: Capillary LC, HPLC, Mass Spectrometry, Proteomics
Application Code: Genomics, Proteomics and Other 'Omics
Methodology Code: Liquid Chromatography/Mass Spectrometry
Injection is one of the most important processes for fast and ultra-fast GC analysis. A narrow band start peak is essential for a high quality, fast GC separation. The most common method to obtain a narrow band peak is split injection. However, this method has a reduced amount of sample injected into the system, consequently reducing the overall method sensitivity. The other drawback of the split method is that it may cause sample discrimination which will affect the sample quantification. Another method to obtain a narrow band is the use of cryogenic refocus technology, but it is only suitable for high boilers. Partial volume injection or dual valves partial volume injection is the other method to achieve a narrow band sample peak, but the amount being injected is influenced by several factors. These factors include timing, flow, pressure, temperature etc. which negatively impacts the repeatability of this method. In this presentation we will use a nano volume valve as the fast and ultra-fast GC injection system. Since the internal volume and dead volume of the valve are really small (nL level) it can create a sharp and no tailing initial peak without the use of split method or other technology. By using this system, a methane peak with only 8 millisecond peak width at half height has been achieved, which makes it possible to finish a GC run within 1-2 seconds.

Keywords: Capillary GC, Gas Chromatography, GC
Application Code: High-Throughput Chemical Analysis
Methodology Code: Gas Chromatography
New Developments in GC
Optimization of GC Chromatography by Inlet Liner Selection

GC inlet liners can be nearly as important to good chromatography as column selection. Much attention is given to GC columns; their phase selectivity, so-called “Fast GC,” inert and low-bleed varieties. Columns are routinely the primary component discussed when reviewing method development. However, analysts often overlook the much needed, yet forgotten GC inlet liner.

This talk will review the importance of GC liners and demonstrate how given the same GC instrument parameters and column, very different chromatographic responses can be achieved by using different liners.

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<thead>
<tr>
<th>Keywords:</th>
<th>Chromatography, Gas Chromatography, GC, Optimization</th>
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<tr>
<td>Application Code:</td>
<td>General Interest</td>
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<td>Methodology Code:</td>
<td>Gas Chromatography</td>
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New Developments in GC
Simultaneous Multizone Fast Temperature Controls Optimized for Micro GC - Thinking Outside the Airbath Box

With the advent of multizone high speed, high accuracy temperature programming controllers, there are many conventional GC air-bath oven methods that are improved by these more efficient, smaller and faster techniques. These controllers provide opportunity to do temperature programming at very high accuracy, while at the same time very high rates. Reduced mass injectors, vaporizers, transfer lines, concentrators, traps, retention gaps, and columns, can each run in an independent fast rate controlled zone.

This presentation seeks to describe some of the newest devices and controls that allow the chromatographer to build method that run considerably better outside the air-bath box.

Keywords: Chromatography, Gas Chromatography/Mass Spectrometry, GC, Thermal Desorption
Application Code: Process Analytical Chemistry
Methodology Code: Gas Chromatography
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<th>Session Title</th>
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<tr>
<td>Abstract Title</td>
<td>A GCxGC Flow Modulator with Alternate Primary Column Flow Direction for Long Secondary Separation Time</td>
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<tr>
<td>Primary Author</td>
<td>Huamin Cai</td>
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<td>Valco Instruments Co. Inc.</td>
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**Abstract Text**

Flow modulators have been used for GCxGC separation for many years. Compared to the cryogenic modulator, it has the advantages of simplicity, low cost, and high using temperature. However, the flow modulator usually has short secondary retention time because it needs to maintain continuous primary column flow. In this presentation we will propose an alternate primary flow direction method to increase secondary retention time. This method uses a valve to make the primary column flow reversed during secondary separation. Consequently it makes the sample stay in the primary column longer and gain more time for secondary separation. By using this method a 20-second secondary retention time has been achieved with 100% transfer from primary to secondary. A longer secondary retention time can increase the secondary separation power.

**Keywords:** Gas Chromatography, GC

**Application Code:** General Interest

**Methodology Code:** Gas Chromatography
A Recently developed, free to use, web-based computer program has been employed to accelerate Gas Chromatography (GC) method development by direct simulation of the chromatographic process. The program makes use of a highly accurate time-summation modeling approach, coupled with large libraries of data to provide absolute retention time predictions within 1% of those obtained from experiment.

The user is able to directly control every parameter of the chromatography being modeled including carrier gas, stationary phase, column dimensions, and temperature program. The temperature program can be iteratively optimized to obtain the desired separation in the minimum run time. The software is preloaded for modeling of more than 20 different stationary phases and hundreds of compounds including, Pesticides, PCB’s, PAH’s, semivolatile, and volatile organics.

These GC modeling features will be discussed with a focus on experimental agreement for both atmospheric and vacuum outlet GC, modeling in the context of method development, and modeling of very challenging separation problems. Our goal is to make computer modeling of GC separations an intuitive and easily accessible tool in laboratory workflows.
Chromatographic resolution of complex mixtures and closely stereoisomers is one of the most challenging tasks in modern pharmaceutical analysis. Two-dimensional chromatography is often applied to increase peak capacity and selectivity by combining two orthogonal chromatographic conditions in a single chromatographic run. In this presentation we illustrate how ultrafast chiral chromatography in the second dimension can be successfully applied to the separation and analysis of complex mixtures of closely related stereoisomers and regioisomers.
Ion Pairing as the Main Pathway for Reducing Electrostatic Repulsion Among Organothiolate Self-Assembled on Gold Nanoparticles in Water

Abstract Text

Organothiol binding to gold nanoparticles (AuNPs) in water proceeds through a deprotonation pathway in which the sulfur-bound hydrogen (RS-H) atoms are released to solution as protons and the organothiol attach to AuNPs as negatively charged thiolate. The missing puzzle pieces in this mechanism are (i) the significance of electrostatic repulsion among the likely-charged thiolates packed on AuNP surfaces, and (ii) the pathways for the ligand binding system to cope with such electrostatic repulsion. Presented herein are a series of experimental and theoretical evidences that ion pairing, the coadsorption of negatively charged thiolate and positively charged cations, is a main mechanism for the system to reduce the electrostatic repulsion among the thiolate self-assembled onto AuNP surfaces. This work represents a significant step forward in the comprehensive understanding of organothiol binding to AuNPs.

Keywords: Infrared and Raman, Surface Enhanced Raman Spectroscopy, UV-VIS Absorbance/Luminescence, Vibrational Spectroscopy
Differential Scanning Calorimetry (DSC) is widely used to characterize polymers based on their heat flow behaviors - both exothermal and endothermal - upon heating and cooling. Until recently, identifying unknown polymers based on their DSC profile was done manually, a cumbersome and knowledge-intensive process. This presentation will introduce a unique new thermal analysis databasing software known as IDENTIFY and apply it to the DSC traces of several popular kitchen utensil scrubbers of unknown composition in order to determine their polymer makeup.

The IDENTIFY software system uses an approach very similar to that employed by modern image recognition software for identifying persons or objects. This approach can be divided into three main tasks:

1. Segmentation of the DSC measurement curve: Significant caloric effects like glass transitions or exothermic- and endothermic effects must be identified and distinguished from irrelevant parts of the DSC curve.
2. Extraction of the properties of the effects found: Properties such as extrapolated onset temperature or peak area are evaluated automatically according to known DIN or ASTM E standards using classical features.
3. Recognition of the DSC curve: The unknown DSC curve is compared not only to specific database measurements and literature data but also to classes. Similarity values are computed practically in-situ using advanced mathematical algorithms. An optional selection of the temperature range allows for example for restriction to only certain DSC effects. Algorithm types for single- or multi-component samples as well as different parameter setups are available which would take into consideration any additional information on the sample.

Identify can search through hundreds of database entries and – within a single second – find the DSC measurement curves most similar to the unknown curve.

Keywords: DSC, Materials Characterization, Polymers & Plastics, Thermal Analysis
Application Code: Polymers and Plastics
Methodology Code: Thermal Analysis
Existing studies on molecular fluorescence have almost exclusively been focused on Stokes-shifted fluorescence spectroscopy (SSF) in which the emitted photon is detected at the wavelengths longer than that for the excitation photons. Information on fluorophore on-resonance fluorescence (ORF) and resonance Rayleigh scattering (RRS) is limited and often problematic due to the complex interplay of the fluorophore photon absorption, ORF emission, RRS, and solvent Rayleigh scattering. Reported herein is a relatively large-scale systematic study on fluorophore ORF and RRS using the conventional UV-vis extinction and SSF measurements in combination with the recently reported ratiometric resonance synchronous spectroscopic (R2S2, pronounced as “R-Two-S-Two”) method. A series of fundamental parameters including fluorophore ORF cross-sections and quantum yields have been quantified for the first time for a total of twelve molecular and six semiconductor quantum dot (QD) fluorophores. All fluorophore spectra comprise of a well-defined Gaussian peak with a full width at the half maximum ranging from 4 nm to 30 nm. However, the RRS features of fluorophores differ drastically. The effect of fluorophore aggregation on its RRS, UV-vis, R2S2, and SSF spectra was also discussed. This work highlights the critical importance of the combined UV-vis extinction, SSF, and R2S2 spectroscopic measurements for material and characterizations. The method and insights described in this work can be directly used for improving the reliability of RRS spectroscopic methods in chemical analysis. In addition, it should pave way for developing novel R2S2-based analytical applications.

Keywords: Fluorescence, Light Scattering, Method Development, UV-VIS Absorbance/Luminescence
Application Code: Bioanalytical
Methodology Code: Fluorescence/Luminescence
X-ray diffraction is one of the most commonly used techniques for structural characterization of both inorganic and organic materials in powder form along with other spectroscopy techniques such as FT-IR and Raman. However, studies on materials for their physico-chemical properties as a function of temperature, environment, pressure and other conditions require dynamic crystallographic measurements in real time. Indeed, structural phase transitions or modifications of materials can be captured as they occur in real-time thanks to Position Sensitive Detectors (PSD). We have developed an X-ray diffraction portfolio using PSDs from simple bench-top instruments to the most advanced platforms which enable material scientists and engineers to perform qualitative, quantitative and advanced structural investigations on a variety of materials. This presentation will cover various applications from routine QC/QA related phase quantification in industrial process control to real-time determination of structures, texture, residual stress, polymorphism, reactivity or kinetics of advanced materials in the form of powders or thin films. Together with the most advanced data processing software programs based on Rietveld, we will present the advantages of such unique XRD capabilities.

**Keywords:** Analysis, Characterization, Material Science, X-ray Diffraction

**Application Code:** Material Science

**Methodology Code:** X-ray Techniques
This presentation introduces the spectroscopic concepts and recent 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 very recent 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: Characterization, Chemometrics, Portable Instruments, Spectrometer
Application Code: Homeland Security/Forensics
Methodology Code: Sensors
Dielectrophoretic Manipulation of Individual Ag and Pt Nanoparticles and Their Stochastic Electrochemical Detection

Methods of stochastic electrochemical detection, developed by Bard and Compton groups, allow for determination of individual analyte species such nanoparticles (NPs), protein and DNA macromolecules, liposomes and viruses, and even ions.

Here we are presenting the results of our investigations, in which we employed the use of dielectrophoretic force for preconcentration of analytes such as Ag and Pt NPs. The objective of this research is to demonstrate the feasibility and advantages of dielectrophoresis (DEP) compared to other methods of analyte preconcentration. We anticipate that by using DEP for analyte manipulation, it will be possible to not only decrease the detection limits, but also improve the specificity of the stochastic electrochemical detection since only analytes experiencing positive DEP (i.e., moving towards the indicator electrode) will be detected.

The experimental setup included a three-electrode cell. The working electrode was either a carbon fiber UME, 11 m diam. (used in the detection of Ag NPs) or a Au UME, 9 m diam. (used in the detection of Pt NPs). The alternating current (ac) signal, that caused the appearance of the DEP force on the metal particles, was applied between the working and counter electrodes.

The results in Figures 1 and 2 clearly indicate a substantial increase in the frequency of NP collisions upon application of the ac signal. For both cases (Ag and Pt NPs) the frequency of collisions has increased by at least an order of magnitude because of the DEP effect.

Obtained results are the first examples of the stochastic electrochemical detection of analytes, in which the DEP force has been applied for the analyte preconcentration at the electrode surface. It is expected that the developed technology will be particularly useful for the analysis of ultra-low concentrations of various analytes, many of which can be disease and warfare agents.

This work has been supported by the University of Akron start-up funding to A.B.

Keywords: Detection, Electrochemistry, Microelectrode
Application Code: General Interest
Methodology Code: Electrochemistry
Petrochemical Analysis by GC (Half Session)

Rapid Analyses Condition Monitoring for Fuel and Antifreeze in Used Engine Oil

Two separate analyses will be presented for the determination of contaminants in used engine oil by gas chromatography. The presence of fuel in used engine oil can be caused by leaking valves, piston rings or fuel injectors. The presence of ethylene glycol in used engine oil is an indicator of engine coolant leaking into the engine crankcase past sealing gaskets. The routine analysis for these contaminants can predict engine problems allowing for preventative maintenance before engine failure causes more costly consequences.

High throughput screening for fuel dilution in used engine oil is accomplished by direct injection into a gas chromatograph with no sample preparation. Low resolution chromatography, isothermal separation and backflush of the high molecular weight matrix allows for a 2 minute analysis time or about 400 sample per day capability. Diesel is the primary fuel of interest but the method can also differentiate between diesel, gasoline or biodiesel fuel.

Antifreeze analysis in used engine oil employs headspace extraction into a gas chromatograph. In situ derivatization is used for the ethylene glycol in antifreeze to increase volatility and reduce polarity for easy chromatography. Flame ionization detection provides sufficient sensitivity to low ppm levels while selective detectors can be used for sub-ppm levels. Rapid analysis of about 1 minute per sample will allow for large volume throughput of 400+ samples per day.

Both methods are now peer reviewed and accepted as official ASTM methods.

Keywords: Fuels\Energy\Petrochemical, Gas Chromatography, GC
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Gas Chromatography
This presentation will discuss new methodology to assess trace components that impact the quality of retail Liquefied Petroleum Gas (LPG). Dissolved water in LPG can cause freeze-up difficulties in pressure reducing systems, leading to safety, corrosion and operational issues in LPG distribution systems. Methanol is sometimes added to the LPG to mitigate water contamination issues. Retailers need to know the water content of LPG to determine how much methanol to add without exceeding the methanol specification limit.

The current industry practice is to measure water content indirectly using a valve freeze test (ASTM D2713) in order to determine specification compliance. Refiners and producers of LPG must meet a specification limit of “pass” using this method. The procedure shows poor repeatability, requires venting to atmosphere of relatively large amounts of LPG vapor, and is subject to interference from antifreeze agents (methanol). An LPG sampling unit for quantitative determination of water in LPG by Karl Fischer titration is developed but is subject to interference from mercaptans (odorant). Industry has a need to identify an alternate method for accurate measurement of water content in LPG.

The use of ionic liquid column with gas chromatography vacuum ultraviolet detector (GC-VUV) for separation and quantitation of water, methanol and ethyl mercaptan in LPG will be presented.

Keywords: Fuels\Energy\Petrochemical, Gas Chromatography
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Gas Chromatography
While incremental advancements continue to be reported in microchip gas chromatography (µGC), the current performance is far from acceptable. The reasons are largely attributed to factors such as shorter column length (limited peak and sample capacity), presence of active sites (increased adsorption of sensitive compounds), non-uniform stationary phase coating (pooling at points of high curvature) and inadequate interfacing technology (limited temperature stability and detrimental dead volume). These shortcomings can be mitigated by applying temperature programming of the microchip in a unique way.

First reported by Zhukhovitskii in the 1950s, thermal gradient gas chromatography (TGGC), in which a negative gradient is applied along the column can be used to correct for non-ideal chromatographic conditions.

In this work, µGC columns were fabricated, coated and interfaced to injector and detector in a novel compact design. This design utilized two heaters: the first was used to generate the desired gradient profile, while the second was used to raise the temperature for compound elution. The TGGC design eliminated many of the shortcomings of µGC and allowed analysis above 300°C.
Ionic liquids (ILs) containing long alkyl side chain substituents have been previously applied as stationary phases in comprehensive two dimensional gas chromatography (GC × GC) for the selective separation of nonpolar analytes in kerosene. However, the high melting points of these ILs limit the separation of analytes at low operating temperatures. In order to address this issue, a series of lipidic ILs containing long-chain substituents were examined as stationary phases in this study. Compared to their saturated homologues which are solid at room temperature, lipidic ILs possess lower melting points and exhibit better selectivity toward nonpolar aliphatic hydrocarbons in GC × GC separations. The solvation properties of four selected lipidic ILs and two saturated homologue ILs were evaluated using the Abraham solvation parameter model. The presence of functional groups (i.e., double bonds, sulfur heteroatoms, and cyclopropanated structure) affected the hydrogen bond basicity, hydrogen bond acidity, as well as dispersive interactions of the ILs. This study provides the first comprehensive examination into the relation between the structure of lipidic ILs and the resulting solvation characteristics. The results show that lipidic ILs containing various functional groups can be interesting candidates for solute specific separations in GC × GC.
A novel MS calibration has been shown to enable accurate mass and elemental composition determination with quadrupole GC/MS. To get structural information, however, one typically searches a compound library such as NIST library, which was created with only nominal masses. In this paper, we will present a new approach to search conventional mass library with accurate mass and create customized libraries with accurate masses for higher confidence identification. A new data-driven approach will also be presented to analyze various fragments, even when they are spectrally overlapped, to aid in the structural elucidation.

Test samples are acquired in the “Raw Scan” (profile) mode on Agilent 5973 GC/MSD. Towards the end of the run with the GC oven cooled down, the PFTBA tune gas is turned on to acquire standard data to calibrate not only m/z for mass accuracy but also peak shape for spectral accuracy. This calibration would then be applied to each analyte eluted earlier to obtain accurate masses for the identification of molecular ion (if present) or key EI fragments. With the accurate MS available, it is possible to fit the theoretically calculated MS of each possible ions or fragments to the accurate MS to assess the plausibility of the postulated ions or fragments for structural elucidation.

For the compounds tested, all reported accurate masses are within 20mDa mass error with 98.0% spectral accuracy, resulting in the correct elemental composition among the top 3 spectral accuracy hits. The NIST library search can be performed to limit search list only to those with molecular ion exact masses (NIST 2014) within a given mass tolerance or by searching for a given elemental composition in the library or ChemSpider online. A manual approach can also be used to create customized accurate mass library. A high degree of spectral fit (>98.0% spectral accuracy) can be achieved when multiple ions are involved, which can be used to evaluate possible ions and their fragments.
Cytotoxicity of Ionic Liquids – Liposome-Ionic Liquid Interactions Investigated by DSC, DLS, and NMR

The environmentally unfriendly features of some ionic liquids (ILs) have been under much investigation recently. The underlying mechanism of the toxicity and the physicochemical properties of ILs affecting biomembrane interactions are still largely unknown. In this work various analytical techniques were utilized with phospholipid vesicles as model biomembranes to gain information on the cytotoxicity of ILs and the techniques were compared with biological data. The future aim, however, is to be able to collect such data without the need of living cells, organisms, or animals.

The cytotoxicity of some biomass-dissolving ILs was assessed by measuring the median effective concentrations using human corneal epithelial cells and Vibrio fischeri 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 rupturing data. Differential scanning calorimetry on dipalmitoylphosphatidylcholine vesicles was used to get information on the penetration of the ILs into the lipid bilayer. The effect of ILs on the size and the zeta potential of negatively charged phospholipid vesicles (liposomes composed of phosphatidylcholine and phosphatidylglycerol) were assessed in order to obtain information on the changes in the liposome bilayer. Furthermore, in order to study the physical state of the IL before and after liposome bilayer rupture, pulsed field gradient nuclear magnetic resonance spectrometry was utilized. The result show that the investigated ILs can be divided into two groups based on the cytotoxicity mechanism: cell rupturing ionic liquids and cell wall penetrating ionic liquids.

Keywords: Light Scattering, Lipids, NMR, Toxicology
Application Code: Clinical/Toxicology
Methodology Code: Chemical Methods
### Abstract Text

Indirect nanoplasmonic sensing (NPS) is a novel microanalytical technique based on the optical phenomenon of localized surface plasmon resonance of metallic nanostructures. In indirect NPS experiments the plasmonic particles are inert and adjacent to the material of interest to probe a process occurring either in the material investigated or on the surface of the material. Changes in the nanoparticle properties cause a shift in the maximum-extinction wavelength, which can be monitored and recorded in real-time. Among the notable advantages of NPS are the simple instrumentation and the extremely robust and noninvasive methodology, which is very flexible regarding the type of material investigated. Recently, NPS has been successfully applied to the development of biosensors, biochemical processes, and to the binding characterization of polymers, involving kinetic measurements.

The increasing use of ionic liquids in industrial processes has resulted in strong demands on achieving a better understanding of the toxicological effects of ionic liquids. There is a great demand for more systematic studies on the effect of ionic liquids on biological membranes and in this work we have employed phospholipid vesicles as biomimetic models.

Large unilamellar synthetic phospholipid vesicles were prepared and immobilized on different hydrophilic sensors (titanium dioxide and silicon nitride surfaces) and the influence of industrially relevant ionic liquids on the adsorption pattern was studied by NPS. The effect of ionic liquids on phospholipid vesicles was shown to be highly dependent on the hydrophobicity and molecular structure of the ionic liquid. In general, this project will lead to a better understanding of the possible harmfulness of novel ionic liquids on phospholipid vesicles, acting as excellent artificial models for biomembranes.

**Keywords:** Bioanalytical, Immobilization, Lipids, Sensors

**Application Code:** Bioanalytical

**Methodology Code:** Chemical Methods
A fundamental problem with efficiency in capillary action driven planar chromatography results from diminishing flow rates as development proceeds, giving rise to molecular diffusion related band dispersion. Overpressure and electrokinetic means to speed flow are used successfully in thin layer chromatography (TLC). Herein we explore the use of centrifugal force (CF) as a means to drive flow for reduced-dimension planar platforms (ultra-TLC, low [micro]m features, and nano-TLC, nanoscale features). The silicon wafer platforms have two forms of continuous 2-D arrays created by either photolithography or metal dewetting followed by deep etching and made to have a porous SiO[sub]2[/sub] shell. The flow pattern is unusual with co-planar flows above and within the arrays. The effects of parameters such as spin rate, solvent type, and surface character on flow rates is established and can be substantially greater than capillary action flows. Using fluorescent dyes, we investigate retardation factors and chromatographic plate height; the latter falls in the low to sub-[micro]m range. To the best of our knowledge, we demonstrate herein the first analytical separations performed in pillar arrays using centrifugal force to augment solvent flow.

Keywords: Chromatography, Lab-on-a-Chip/Microfluidics, Separation Sciences
Application Code: Clinical/Toxicology
Methodology Code: Separation Sciences
A long alkyl group like C30 (triacontyl group) phase has been known to be more suitable than a conventional C18 phase for separation of hydrophobic structurally related isomers such as vitamin E or vitamin K1. In this study, separation factor of beta-tocopherol and gamma-tocopherol which were structurally related isomers was evaluated to vary both a pore diameter of the superficially porous silica and a ligand density of the C30 group. Regarding a pore diameter, 12 nm showed the largest separation factor of beta and gamma-tocopherol among 10 nm, 12 nm and 16 nm. Regarding a ligand density, the higher a ligand density, the larger a separation factor of beta and gamma-tocopherol. However, when a ligand density was too high, much high hydrophobicity caused a peak tailing and a drop of theoretical plate. The most suitable ligand density existed for the highest resolution. Finally separation of cis and trans-vitamin K1 was compared and the same result as separation of beta and gamma-tocopherol was obtained.
Contrast-enhanced computed tomography (CT) and spectral (color) X-ray CT have the potential to enable molecular imaging in CT. However, there is a lack of contrast agents designed to fully leverage the capabilities of spectral CT. Therefore, the objective of this study was to develop a modular approach to design a spectral library of core-shell nanoparticle (NPs) contrast agents, which will have broad applications in biomedical imaging due to potential for multi-modal imaging (e.g., fluorescence, MRI, X-ray, plasmonic resonance), dosed delivery of therapeutics and active targeting through molecular surface functionalization. 12-15 nm Gd\(_2\)O\(_3\), HfO\(_2\) and Au core compositions were prepared using solution phase synthesis or a templated approach. Silica shells of controlled thicknesses (1-15 nm) enabled predictable loading of fluorescent molecules and provided a common platform for molecular surface functionalization. Antibodies and other small molecules were covalently conjugated to the nanoparticles and the bioactivity and orientation of IgG antibodies conjugated to NPs were confirmed through agglomeration assays and electron microscopy. The multi-modal bioactive NPs were then successfully targeted to ovarian cancer cells associated poor prognosis in ovarian cancer patients and also HER2+ breast cancer cells, which are found in ~30% of breast cancer cases worldwide. Both cell lines were targeted with high binding specificity in vitro and factors affecting intra-cellular distribution of NPs were analyzed using appropriate statistics. Finally, a xenograft model of ovarian cancer was constructed using cd133(+) SKOV3-IP cells in a nude mouse model. The tumor location in vivo was determined from the fluorescence and X-ray attenuation signal associated with the anti-cd133 tagged multi-modal NPs probes. The results from this study lays the foundation for modular assembly of targeted theragnostics agents.
Chemical analysis' accuracy and precision is dependent upon a calibration graph and its associated errors. Calibration graphs commonly use the least squares regression model for data analysis. The least squares regression assumes homoscedastic noise but research shows that noise is rarely homoscedastic, especially when the calibration graph spans many decades of concentration (such as in ICP-OES). Heteroscedastic noise is better modeled with weighted least squares regression. The weighted least squares model is more accurate but it requires knowledge of each point in the calibration graph. A large number of replicates are required to estimate the precision with high confidence. Magnitudes of noise sources vary greatly along with calibration graph as concentrations change. We present noise source characterization for ICP-OES along with a model developed to effectively predict the standard deviation of emission as a function of concentration.
Detection of Endocrine Disruptors Using Male Blacknose Dace (Rhinichthys Atratulus)

Xenoestrogens (or estrogen mimics) are described by the US EPA as “Contaminants of Emerging Concern” both because of their impact on aquatic life and because of their potential impacts on humans. Detection and quantitation of xenoestrogens is challenging because they include a wide range of chemicals and that have been demonstrated to be dangerous even at concentrations in the low parts per trillion. Xenoestrogens are known to stimulate the males of several fish to produce ova in their testes. We describe a project in which we use Blacknose Dace (Rhinichthys atratulus) as a bioassay preparatory to designing strategies for chemiluminescent analysis of the water for xenoestrogens. We chose three streams in the watershed of Seneca Lake (in the Finger Lakes region of New York state), and we describe microscopic analysis of the histology of gonads from male Blacknose Dace. We have analyzed over 100 Dace for testis-ova, and the preliminary results for the histology of the gonads suggest that the sampled streams are relatively free of xenoestrogens. Testis-ova were found in only a single stream, where they occurred at a low frequency and at a low severity.

Keywords: Chemiluminescence, Environmental/Water, Microscopy
Application Code: Environmental
Methodology Code: Microscopy
Core-Shell particles have become more and more popular in HPLC, finding great use in allowing speed to be increased, high resolution to be achieved and sensitivity to parallel that of UHPLC particles, but without the increased backpressure associated with UHPLC.

If you wish to run fast analysis then the use of particles with higher efficiency allows the resolution between peaks to be increased and therefore speed to be decreased significantly. However the use of efficiency alone is not the strongest term in the resolution equation and cannot be relied upon for more complex separations with critical pairs.

In this poster we discuss the use of a new core-shell polar embedded stationary phase. This new phase shows orthogonal selectivity to that of alkyl chain stationary phases, allowing the analyst the ability to obtain high resolution between critical pairs. We show applications of selectivity using simple mobile phase’s but utilising the separation power of the polar embedded group to achieve the required resolution.

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

**Application Code:** General Interest

**Methodology Code:** Separation Sciences
Biomarkers are specific compounds which can be used to identify archeologically and anthropologically significant ingredients believed to be used in ritual ceremonies and other cultural practices. Various biomarkers can persist in the inner pores of many pottery vessels and other artifacts for extended periods of time. For example, tropane alkaloids such as scopolamine in jimson weed (datura stramonium), were used in many ritualistic practices and often mixed with other plants and foods during preparation of ceremonial beverages. Biomarkers can be characterized using modern instrumentation such as gas chromatography- mass spectrometry (GC-MS) and liquid chromatography- mass spectrometry (LC-MS). One important consideration is the potential degradation of biomarkers and the impact of their interpretation on archaeological analyses. Some biomarkers may have degradation products that can likely persist and serve as more suitable markers than the substance originally sought.

**Keywords:** Art/Archaeology, Gas Chromatography/Mass Spectrometry, Liquid Chromatography/Mass Spectrometry
Polyethylene terephthalate (PET) is a polymer commonly used to make soda bottles. One of the monomers, terephthalic acid (TPA), is made by oxidation of p-xylene and dioxygen in acetic acid using a metal catalyst. To ensure high quality PET, the purity of TPA is crucial. This research aims to develop a “green” micellar liquid chromatography (MLC) method to separate and quantify eight impurities commonly found during the production of TPA, including p-toluic, 4-formylbenzoic, isophthalic, phthalic, benzoic, trimesic, trimellitic, and hemimellitic acids.

Previously, we have shown that the concentration of sodium dodecyl sulfate [SDS] in the mobile phase both below and above the critical micelle concentration for SDS (0.23%) can markedly affect the retention of aromatic acids. In addition, pH is an important factor because more neutral acids should interact more favorably with the hydrophobic stationary phase. Using a standard HPLC C-18 column, plots of retention factor (k') versus both increasing [SDS] from 0.1 - 2% and pH at 3, 4, and 6.5 tended downward. Baseline separation of the three phthalic acids is possible in about 6 min and that for the three tricarboxylic acids in less than 2 min. In general, the best mobile phase for the separation of seven acids appears to be 1 - 2% SDS at pH 3. Benzoic and p-toluic acids, being the most hydrophobic, were irreversibly retained but a flowrate/pressure step gradient has been found effective to promote elution. Adaptation to the UHPLC mode is being continued.
Cold EI is electron ionization of vibrationally cold molecules in supersonic molecular beams (SMB) with a fly-through EI ion source. The Aviv Analytical 5975-SMB GC-MS with Cold EI provides enhanced molecular ions, significantly extended range of compounds amenable for analysis, much faster analysis and lower limits of detection particularly for difficult to analyze compounds.

GC-MS with Cold EI was converted into electron ionization LC-MS with SMB via the addition to its heated transfer line of a spray formation and vaporization chamber for the conversion of LC output flow into gas phase sample compounds. EI-LC-MS with SMB uniquely provides library based sample identification including at the isomer level, relatively uniform response including for non-polar compounds and absence from ion suppression effects.

Open Probe fast GC-MS was also coupled with the GC-MS with Cold EI for the provision of real time analysis with 30 seconds separation and under one minute full analysis cycle time. The Open Probe Fast GC-MS can operate with any GC-MS but it excels with GC-MS with Cold EI in view of its enhanced molecular ion, tailing free fast GC peaks and extended range of compounds amenable for analysis.

We also developed improved sample identification software named TAMI that provides improved quadrupole mass accuracy and combined with isotope abundance analysis it provides elemental formulae and is automatically linked with the NIST library to further improve sample identification by mass spectrometry with Cold EI.

**Keywords:** Gas Chromatography/Mass Spectrometry, Liquid Chromatography/Mass Spectroscopy, Ultratrace An

**Application Code:** General Interest

**Methodology Code:** Mass Spectrometry
Organophosphorus pesticides are usually used to increase crop yield. Nevertheless, their residues in agricultural products and contamination of water exhibit acute toxicity, which inhibit the activity of acetylcholinesterase resulting in accumulation of the neurotransmitter acetylcholine in the body and thus inflicting serious harm to the nervous system. Therefore, determination of OPPs is highly desirable. One of the most important approaches for the determination of pesticide residue is high performance liquid chromatography along with dispersive liquid–liquid micro-extraction (DLLME). Ionic liquids (ILs) have been introduced into DLLME as extraction solvents. ILs are considered “green solvents” due to their negligible vapor pressures, good thermal stabilities, can solubilize a variety of organic and inorganic compounds, and adjustable miscibilities through designing the structure of ILs. In this study, based on the similar structure between carbonic acid ester and phosphate ester, a functionalized ionic liquid, 3-Methyl-1-(ethoxycarbonylmethyl)-imidazolium bis(trifluoromethylsulfonyl)imide ([MimCH2COOCH3][NTf2]) was synthesized and employed as extraction solvent for the determination of organophosphorus pesticides (OPPs). The extraction procedure was induced by the formation of a cloudy solution, which was composed of fine drops of [MimCH2COOCH3][NTf2] dispersed entirely into the sample solution with the help of disperser solvent (acetonitrile) to achieve rapid extraction in two minutes. After the optimization of extraction conditions, the enrichment factors were approximately 400. The detection limits were in the range of 0.66-10.25 nmol/L. The developed method was successfully employed for the analysis of OPPs in environmental water samples, which exhibited excellent application prospect in the environmental field, and it is significant to design the ionic liquids based on the different structure of target analytes for extraction.

Keywords: Chromatography, Environmental Analysis, Extraction, Method Development
Application Code: Environmental
Methodology Code: New Method
The use of microfluidics has shown rapid growth with the development of the so-called “laboratories on a microchip” (lab-on-a-chip), which are devices that have integrated laboratory functions, such as analysis and mixing of reagents and separate products in the same microdevice. Polyphenols in olive oil have been studied because of high antioxidant value. There are many methods for their extraction but these are very laborious, use toxic reagents and the analysis time is long, therefore it is necessary to develop methodologies more efficient and rapid. A microdevice, constructed by soft lithography, for the liquid-liquid extraction and spectrophotometry quantification of polyphenols from olive oil was evaluated. The microchips consisted of two part: 1) a coil of 26.36 cm to extract polyphenols from oil using a 0.75 mol/L pH 10.6 carbonates solution and 2) a coil of 10.75 cm to develop the reaction product using Folin-Ciocalteau reagent. Reagents and sample were introduced into the microchip at 40 µL/min using a syringe pump. Detection of the aqueous product involved off-line coupling of a Z shape flow-cell right at the microchip outlet where optical fibers were inserted. The lineal range was from 2.0 to 20.00 µg/mL for tannic acid. The intra-lab repeatability and reproducibility were 1.45 % and 2.93 %, respectively. The extraction and quantification was carried out in 6 min. The method was compared with the standard method proposed by International Olive Council (IOC) being the extraction efficiency by the proposed microchip method higher (47 % more) than by the IOC method.

Keywords: Extraction, Food Identification, Food Science, Lab-on-a-Chip/Microfluidics
Application Code: Food Science
Methodology Code: Microfluidics/Lab-on-a-Chip
Fluorescence detection is widely used in analytical and bioanalytical science. In microfluidics field, fluorescence is a primary tool for on-chip detection. Although single-molecule detection systems can be assembled, these require additional expertise and can be relatively expensive. Recently, we designed a passively operated microfluidic device (µChopper) consisting of two directly opposing aqueous channels that intersected with an oil channel to form droplets in an alternating fashion. Picoliter volume sample and reference droplets were alternatively passed by a detector, allowing significant reductions in system noise and enabling sensitive absorbance detection. This approach thus provided a practical means for absorbance detection on-chip without complex optics. Here, we extend the µChopper concept to on-chip fluorescence detection with standard microscope optics, providing a practical way to reduce noise in a more typical microchip detection setup. We first improved the precision of droplet control in the µChopper by adding normally-closed pneumatic valves that enabled highly precise, automated control of droplet formation, and fluorescence emission measurement could be phase locked with the droplets via computer control (LabVIEW). Using buffer in reference droplets, the system exhibited a limit of detection (LOD) of 5 pM for fluorescein. When 10 nM fluorescein was used as a reference, differences as small as 240 pM could be resolved between reference and sample droplets. Finally, we used the µChopper to quantify free fatty acid (FFA) uptake in 3T3-L1 adipocyte cells. Uptake of fluorescently labelled palmitate (BODIPY-C12) was quantified after lysing between 1-10 cells. Single-cell FFA uptake was resolvable, and uptake rates were measured to be $3.5 \pm 0.2 \times 10^{-15}$ mol cell$^{-1}$. The combination of this automated µChopper with lock-in detection provides a high performance platform for detecting small differences in standard fluorescence detection setups.
## Abstract Text

Proximity immunoassays (PIA) are recently developed protein detection techniques with high selectivity and sensitivity; examples include proximity ligation assays (PLA), electrochemical proximity assay (ECPA), Förster resonance energy transfer assays (Pincer Assay), proximity extension assays (PEA), etc. PIAs take advantage of the “proximity effect” of two antibody-oligonucleotide conjugates (Ab-N) or aptamer pair probes that recognize a target molecule simultaneously. The proximity effect induces target-molecule-dependent hybridization of oligonucleotide tails of the bound probes, which triggers downstream reactions for signal amplification or direct readout. Independently, nucleic acid templated reactions have recently been applied in DNA or RNA detection or in cell imaging, where a reaction between custom labeled nucleic acid probes can be triggered by hybridization to the nucleic acid target. Fluorogenic nucleic acid detection was achieved by converting a pro-fluorophore into a fluorophore, removal of covalently attached quencher, or de novo fluorescent dye generation. In this work, we report the synthesis of probes that will be used in proximity effect-induced, nucleic acid templated, azide-caged fluorophore Staudinger reduction for eventual protein quantification. 4-Bromo-1,8-naphthalic anhydride was treated with beta-alanine to introduce a carboxylic acid group for DNA conjugation, followed by azidization to achieve the azide-caged fluorophore. The reduction reagent was prepared from tris(2-carboxyethyl)phosphine (TCEP) by esterification. The azide-caged fluorophore and the reduction reagent were covalently linked with separate DNA strands by the EDC-NHS reaction. Upon hybridization and reduction, the fluorescent intensity increased 7.5-fold. These probes will be combined with antibody-oligonucleotide conjugates for protein detection using proximity-effect templated synthesis of a fluorophore.

## Keywords
- Bioanalytical
- Biosensors
- Fluorescence
- Nucleic Acids

## Application Code
- Bioanalytical

## Methodology Code
- Fluorescence/Luminescence
In this study, Artemia franciscana (brine shrimp) were used as a model organism to explore the effects of exposure to sublethal levels of tris(1,3-dichloroisopropyl)phosphate (TDCIPP), a high-production volume organophosphate-based flame retardant (OPFR) widely used as a PentaBDE replacement. TDCIPP is of particular interest due to its prevalence in the environment\(^1\) and toxicity to aquatic organisms.\(^2,3\) Artemia exposed to TDCIPP were fixed in 384 well plates and analyzed using the ImageXpress Micro Widefield High-Content Screening System. TDCIPP concentrations that have an effect on the body area of the developing naupliar Artemia were targeted for further study. 1H NMR and GC-MS were used in metabolomics analysis of Artemia extracts to identify metabolite biomarkers of exposure. Using three complementary analytical platforms, we hope to establish Artemia as a model organism for pollutants in saltwater ecosystems and to further elucidate the impact of TDCIPP in aquatic invertebrates.


Keywords: Environmental, Imaging, Metabolomics, Metabonomics
Application Code: Bioanalytical
Methodology Code: Magnetic Resonance
Gold nanoparticles have been established as an ideal signal enhancement tool in a wide variety of sensing schemes and detection modalities. We report a novel conjugation strategy based on DNA linkers and diluents in place of conventional self-assembled monolayers. For model biotin-streptavidin systems, the nanoparticles were assembled through thiol-gold bonding of ssDNA and salt aging, with pre-conjugated biotin moieties facing outward from the gold surface. These nanoparticles are shown to be highly stable under a wide range of buffers and ionic strengths, in contrast to other functionalized gold colloids. They were then applied in a signal enhancement scheme for surface plasmon resonance (SPR) imaging of membrane receptor interactions, based on nanoparticle mediated biotin and nucleic acid assembly processes. Phosphocholine vesicles were fused into fluid supported lipid bilayers on nanoglassified gold sensor chips. The bilayer membranes were initially incorporated with the cell surface ligand GM1 for cholera toxin capture and sensing. Following an initial amplification through a biotinylated detection antibody, a streptavidin bridge linked a biotin and DNA modified gold nanoparticle. In addition to plasmonic coupling based amplification with the underlying gold surface, these DNA-tagged materials enable additional enhancement strategies to be applied through enzyme free amplification schemes. Furthermore, the high stability has allowed for multiple reactions and conjugations to be performed within the colloidal suspensions (i.e. Protein A and antibody binding) for tailored and specific interactions to take place. Together, these properties render this material suitable for clinical, field, and point-of-care applications where stability and extended shelf lives are greatly needed.

Keywords: Biosensors, Nanotechnology, Nucleic Acids, Spectroscopy
Application Code: Nanotechnology
Methodology Code: Sensors
The colon was long thought of as simply a waste packing organ, but has gained interest with the discovery of the gut microbiome and its importance. Links have been reported between colon health and several diseases including allergies, autism, colorectal cancer, Crohn’s disease, diabetes, and obesity, but the mechanism of metabolic communication is still poorly understood. In this work, use of the Ussing chamber provides a wealth of information on the transport behavior of metabolites including short-chain fatty acids, amino acids, vitamins, and choline derivatives across excised colonic epithelial tissue. These in vitro experiments utilize two chambers separated by epithelial tissue representing both the lumen and serosal sides of the colon. The use of high information content analytical platforms like 1H NMR spectroscopy and GC-MS allows the transport of a wide range of metabolites to be studied. With the addition of 13C-labeled compounds, transformation processes that influence fecal fluid composition during transit across the epithelial tissue can be identified in an effort to gain further understanding of the metabolic processes present throughout the colon.

Keywords: Bioanalytical, GC-MS, Metabolomics, Metabonomics
Application Code: Bioanalytical
Methodology Code: Magnetic Resonance
Isoscapes of the stable light isotopes, primarily hydrogen and oxygen, have been widely used in archaeology, biology, ecology, geology and forensic science. Isoscapes for stable heavy elements such as strontium and lead are less common, in part because they do not as readily fit predictive models for their distribution so that GIS map interpolations can be made. In a recent study (Int. J. For. Sci., 261:83-92, 2016) we developed spatiotemporal lead isoscapes for the United States and Europe for the 19th and 20th centuries based on environmental lead deposition in sediments. In this study, we present the results of MC-ICP-MS analyses of lead concentrations and isotopes in teeth from some 70 modern humans from across the United States who were born and lived in the same location during amelogenesis of their teeth. These results allow an unprecedented analysis of the various source inputs and their on-going impact on lead isoscapes of forensic interest. Funding for this study was provided by the Colorado College Natural Science Division, and by the Otis and Margaret Barnes Trust.
Despite numerous chemical and metallurgical analyses dating back to the 18th century, we still do not fully understand how the ancients managed to mint the quantity of coins that they did, especially the common bronzes of the Roman Empire. Forty-five “Biblical” bronze coins from rulers spanning the first centuries BCE and CE were analyzed using metallography, x-ray diffraction (XRD) and x-ray fluorescence (XRF). These provide thermomechanical information about how coins were minted in ancient Judaea before, during and after the Roman occupation. Based on metallography and a thermal analysis, it appears connected flans were usually struck while they were still hot soon after the metal was poured into chalk-stone molds. This influenced the metal mixture that was chosen for a desired coin mass, though there were also some interesting variations due to other reasons that will be discussed. A chemometric analysis of the texture obtained via XRD indicates reheating of flans before coins were struck became more common during and after the Roman occupation. This suggests that there was a developing specialization in minting methods under the Romans during the first century CE even in the border provinces. Instrumentation used for this study was provided by the Patricia Buster Fund and the Otis and Margaret Barnes Trust.

Keywords: Art/Archaeology, Chemometrics, Microscopy, X-ray Diffraction
Application Code: Art/Archaeology
Methodology Code: X-ray Techniques
Phospholipids are critical components within the membrane of human erythrocytes (red blood cells). When exogenous phospholipids are incorporated into human erythrocytes, a discocyte-to-echinocyte shape change was induced. Under the influence of flippase, the enzyme which transfers specific phospholipids from the outer leaflet to inner leaflet of the membrane, a sub-population of echinocytes shape return back to discocytes again and then become stomatocytes. To better understand the erythrocyte shape change, the change of surface charge on the cell membrane is studied. As an asymmetric charged nanopipette was brought in close proximity to a substrate of interest, the surface charge of the substrate plays a dominant role in the current-voltage response of the nanopipette. Herein, we further extend the application of this method to study the surface charge of human erythrocytes. To better correlate surface charge and cellular shape, high resolution topography images of erythrocytes exposed to phosphatidylserine (PS) were obtained with scanning ion conductance microscopy (SICM) in addition to surface charge measurements at different positions of erythrocytes.


Keywords: Bioanalytical, Electrochemistry, Microscopy
Application Code: Bioanalytical
Methodology Code: Microscopy
Investigations of ion transport properties at nanometer scales is critical for materials science and life science. The use of easy-to-fabricate but powerful nanopipets as local probes has provided promises for measuring ion transport at nanometer scales, especially when coupled with scanning ion conductance microscope (SICM) platform. Potentiometric scanning ion conductance microscopy (P-SICM)[1], a modified configuration of SICM, utilizes a dual-barrel nanopipet probe and significantly increases the signal-to-noise ratio for ion conductance measurements through the potentiometric signal collected at the added barrel. Herein, the differentiation of nearly point-sized transport pathway within an epithelial monolayer, tricellular tight junctions (tTJs), and neighboring longitudinal bicellular TJs (bTJs) pathways is demonstrated with P-SICM for the first time. In this study, P-SICM measurements were conducted in a point-by-point fashion and is limited by the temporal resolution as any external perturbation to drive ion transport can interfere with SICM feedback for probe positioning. We further demonstrate here a customized intermittent “feedback-off” P-SICM approach, which allows the decoupling of feedback control during the application of external perturbation for potentiometric measurements. Simultaneous topography and ion conductance mapping at nanometer scales were achieved with the new approach and applications have been demonstrated with synthetic porous materials as well as biological membranes.

Ion transport across the epithelia can take place in two pathways: the transcellular pathway, in which ions are transported through ion channels in cell membrane, and the paracellular pathway, in which ions are transported through tight junctions formed in the joint of adjacent cells. Potentiometric scanning ion conductance microscopy (P-SICM) has successfully demonstrated the differentiation of the two aforementioned pathways through the measurements of local potential gradient under applied voltage bias to the sample. However, interference arises from the nearby transcellular pathways when probe is positioned over a cell junction, and hence results in inaccuracy in quantitation of junction conductance. In order to address this issue, we proposed a method termed double whole-cell voltage clamp-ion conductance microscopy (DVC-ICM). In DVC-ICM, two patch-clamp pipettes are utilized to patch two adjacent epithelial cells in whole-cell voltage-clamp configuration to hold the cell membrane potential at their resting membrane potential, under which zero current passes the cell membranes. A dual-barrel probe is used to measure the paracellular conductance at the joint tight junction with the adjacent transcellular current eliminated. Results shown here detail the implementation of coupling one patch-clamp electrode to P-SICM and mechanisms of current flow pathways within the system as well the effect of cell patch on both trans- and paracellular conductances.

References.

Keywords: Bioanalytical, Electrochemistry, Microscopy
Application Code: Bioanalytical
Methodology Code: Microscopy
Abstract Text

Scanning electrospray microscopy (SESM) utilizes electrospray from nanopipettes, or pulled glass capillaries with inner diameters that range from tens to hundreds of nanometers, to collect topographic information from a surface. Nanopipettes have been recently developed and characterized as emitters for electrospray ionization mass spectrometry. Further characterization of electrospray current from nanopipettes confirmed a well-known distance-dependent relationship, which has been utilized here as a feedback mechanism for SESM. The distance-dependent current is used as a signal input to a piezoelectric positioner, which correspondingly moves up or down as current changes, to maintain a controlled probe-surface distance. These Z-direction movements are recorded while rastering over a sample surface, and the resultant image corresponds to sample height. Approach curves, line scans, and images are generated over both conductive and insulative features and feature size agrees well with atomic force microscopy of the same substrate. Resolution studies and impact of conductivity on SESM are detailed. SESM provides an ambient, non-contact method to investigate surface topography with initial resolutions in the sub-micrometer regime.

Keywords: Electrospray, Imaging, Method Development, Microscopy
Application Code: Bioanalytical
Methodology Code: Surface Analysis/Imaging
Nanoelectrospray from nanopipettes as emitters have advantages over the conventional electrospray. For example, nanopipettes can produce high charge-to-droplet ratio, have high salt tolerance, require samples in small quantity and can be used to aspirate small amounts of sample as well. It is therefore important to understand the characteristics of the electrospray generated through a nanopipette. We have observed that the size of the emitter plays an important role in defining the electrospray process. The electric field at the tip of the emitter increases with decrease in the tip diameter with all other factors held constant. In our work, pipettes of different sizes ranging between 50nm and 2um were pulled and some of the fundamentals of the electrospray process like flow rates and the electrospray onset potentials were studied. Our results show that the properties of the emitter have a significant effect in various ways on the electrospray produced.

Abstract Text
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Keywords: Analysis, Electrospray, Mass Spectrometry
Application Code: Process Analytical Chemistry
Methodology Code: Mass Spectrometry
Dual-Barrel Ion Channel Probes for SICM

Scanning ion conductance microscopy (SICM) is a non-contact scanning probe technique that can produce sample topography maps of high spatial resolution. However, this technique is limited by an inherent lack of chemical sensitivity. To address the chemical blindness of SICM, our group recently developed a technique we have named ion channel probe-SICM (ICP-SICM). Here, an ion channel (alpha hemolysin) was reconstituted within a lipid bilayer membrane suspended across the opening of an SICM probe. These ICPs displayed a distance-dependent ion current response that allowed for feedback control for imaging, though reconstitution of multiple ion channels within the lipid bilayer was necessary to achieve an ion current of large enough magnitude. Here, we overcome this problem with a dual-barrel pipette in which one barrel serves as the ICP and the other barrel remains open for robust probe-surface distance control. Simultaneous ion current measurements from each barrel result in both the traditional SICM topography map and an additional chemical sensing map from the ICP. We present both approach curves and sample images that demonstrate independent measurements and control of each barrel. Future work with this ICP-SICM system will encompass analysis of biological samples in an effort to better understand hormone/neurotransmitter secretion and possibly relate cellular structure and function with our spatial and chemical maps.

Keywords: Biosensors, Imaging, Lipids, Surface Analysis

Application Code: Nanotechnology
Methodology Code: Surface Analysis/Imaging
Introducing Scanning Electrospray Microscopy to Desorption Electrospray Ionization for Simultaneous 4-D Topographical and Mass Spectrometry Imaging with Nanopipettes

Desorption Electrospray Ionization (DESI), while capable of generating planar chemical images in an ambient environment with little to no sample preparation, currently cannot distinguish height related spatial features on the surface of interest. To achieve this we utilize the distance dependent current generated from the electrospray process as a probe positioning feedback mechanism, as done in Scanning Electrospray Microscopy (SESM). Introducing SESM to DESI is simply accomplished by inserting an ammeter into the DESI setup interfaced through LabVIEW. The end result yields a 3 dimensional surface image with added chemical information correlated through time. Furthermore, unlike traditional DESI which uses fused silica capillaries as a spray probe, we have introduced nanopipettes as our spray probe and nebulizing tip opening up the possibility for new probe designs and quick replacement.

Keywords: Imaging, Instrumentation, Mass Spectrometry, Microscopy
Application Code: General Interest
Methodology Code: Surface Analysis/Imaging
Electrochemical Studies of Carbon Electrodes in Room Temperature Ionic Liquids – Effect of IL Type, Temperature and Electrode Microstructure on Capacitance

The properties of room temperature ionic liquids (RTILs) and the structure of the electrified interfaces they form with carbon electrodes has been the subject of both fundamental and applied research, particularly in the field of energy storage devices like supercapacitors. RTILs have great potential to replace conventional organic solvent/electrolyte systems because of environmentally-benign characteristics (non-volatility, non-toxicity) and thermal and electrochemical stability. The physicochemical properties of RTILs can be flexibly tuned through selection of the component ions. Research is needed to better understand the structure of electrified interfaces formed in these novel media at carbon electrodes of different surface chemistry and microstructure. Traditional models of the electrochemical double layer based on the dilute-solution approximation are not applicable to RTILs because of the absence of solvent, the high concentration of ions, strong interionic columbic forces, and electrostatic and hydrophobic interactions of charged ions with the electrode surface.

The electrochemical investigation of the capacitance of carbon electrodes as a function of potential, the RTIL type, and temperature will be reported. 1-alkyl-3-methylimidazolium- based RTILs were studied at boron-doped-diamond thin-film electrodes. Comparison measurements were made using glassy carbon and nitrogen-incorporated tetrahedral amorphous carbon thin-film electrodes. Cyclic voltammetry and electrochemical impedance spectroscopy were used to measure the electrode capacitance.

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**Keywords:** Electrochemistry, Electrode Surfaces, Temperature

**Application Code:** Other

**Methodology Code:** Electrochemistry
The Cytochrome P450 monooxygenases (P450s) are among the most important catalysts in biology, playing a critical role in oxidizing a wide range of substrates, including sterols, lipids and vitamins. A bacterial enzyme, P450BM3 showed distinct regio- and stereoselectivity towards substrate. There is currently high interest in this enzyme owing to its remarkably high monooxygenase catalytic activity, which mainly results from the fact that it is covalently linked with its cytochrome P450 reductase and this arrangement provides high efficiency electron transfer. Owing to its ability to catalyze oxidation of substrates with high regio- and stereo-selectivity, the P450 BM3 is a popular target of exploration for biotechnologically useful purposes. It has been reported that several mutants can significantly alter the substrate specificity by causing protein conformational changes. Thus, rR studies to probe the heme structure are being performed on a range of mutants of the BM3 heme domain, particularly F87V and A82F, that are known to afford substantial changes in substrate selectivity. Another interesting enzyme is Cytochrome P450 CYP17A1, catalyzes both hydroxylase and lyase reaction along the synthetic pathway of steroid hormones, with the lyase activity being facilitated by cytochrome b5. Though the effects of cytochrome b5 on CYP17 is clear, the mechanism is still in controversial. Cytochrome b5 can function either as an allosteric regulator function or as an alternative electron donor. Thus, Resonance Raman studies are performed on these enzymes to study the protein-protein interactions.

References:
2. Lee-Robichaud, P.; Akhtar, M. [i] Biochem J [i], [b] 1998 [b], [i] 332 [i], 293.

Keywords: Analysis, Bioanalytical, Chromatography, HPLC
Application Code: Drug Discovery
Methodology Code: Vibrational Spectroscopy
The incorporation of monomeric sarcosine oxidase into first generation amperometric biosensor strategies featuring nanomaterials was studied, intended for early stage prostate cancer diagnosis with possible applications for kidney disease and schizophrenia diagnoses. Optimal methods for the production, purification, and stabilization of the enzyme, responsible for the oxidative demethylation of n-methylglycine (sarcosine), were also studied to increase the cost-effectiveness of enzyme production and the overall system. Amperometric measurements of different sarcosine biosensor platforms were analyzed and optimized for sensitivity and selectivity for sarcosine detection across relevant physiological concentrations.

**Keywords:** Biosensors, Detection

**Application Code:** Bioanalytical

**Methodology Code:** Sensors
First generation biosensor development is of interest because the strategy can be readily adapted to many different, clinically-relevant analytes with specific enzymatic reactions to help provide selectivity. Oxidation of hydrogen peroxide, a by-product of said enzymatic reactions, at a platinum electrode allows for amperometric sensing of the targeted analyte in real-time. Layer-by-layer (LbL) xerogel-based biosensor designs have been developed at macro-electrodes where each layer of a specific material serves a function toward sensor optimization. LbL systems have been explored for glucose sensing, as a model system, as well as uric acid sensing for early detection of pregnancy-induced hypertension and preeclampsia risk. In order for such sensors to find practical and effective clinical use, the LbL strategy must be successfully adapted and miniaturized to needle or wire electrodes that can potentially function in vitro as a bedside device, within catheters for continuous, real-time measurements, or as an in vivo implant. Research has progressed via the glucose model to minimize costs and explore how LbL designs need to be adapted when moving to an electrode with a wire geometry. Results of the model glucose LbL system on wire electrodes are presented, which will influence how the strategy can be used for other systems, such as uric acid sensing, which would benefit greatly in clinical applications if successfully implemented at wire electrodes.

Keywords: Bioanalytical, Biosensors, Chemically Modified Electrodes, Electrochemistry
Application Code: Bioanalytical
Methodology Code: Sensors
D-lactate is produced in great excess by Plasmodium, the infectious agent in malaria and can therefore be used as a diagnostic target. A useful diagnostic test needs to meet the World Health Organization's ASSURED standards meaning that it needs to be affordable, sensitive, selective, user-friendly, rapid, equipment-free and delivered to those who need it. Our goal is to meet all of those criteria using a coupled-enzyme assay on a paper microfluidic device that can be analyzed using a cell phone or similar technology. We will present our efforts to insure that this enzyme-based device can be produced, stored, delivered and used by stabilizing the reagents with pullulan, a polysaccharide polymer.
Detection of Hydrofracking Water Infiltration in Surface Waters

Kimberley Frederick
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Ahmed Ismail, Laura Swenson, Tiffany Henao

For each gallon of oil or natural gas that are produced from a hydrofracking well, more water is produced that must be contained and treated or disposed of. This flowback water contains very high concentrations of salts along with radionuclides and organic compounds. Iodide makes a greater early detection indicator for flowback water spills, leaks or insufficiently treated water because the levels are several orders of magnitude higher that those present in uncontaminated surface water. We will detail our efforts to produce a low-cost, user-friendly test for iodide based on a paper microfluidic device with colorimetric detection. We will present our efforts to use two different colorimetric reactions to detect iodide at varying concentrations. Our results on the selectivity, specificity and long term stability of our assay will also be detailed.

Keywords: Environmenta/water, Fuels/Energy/Petrochemical, Lab-on-a-chip/Microfluidics
Application Code: Environmental
Methodology Code: Microfluidics/Lab-on-a-Chip
Enhanced Sensitivity of Inkjet Printed Sensors by Electrochemical Metal Deposition

Inkjet printing has recently become popular to fabricate low cost devices for electrochemical biosensing and bioanalytical applications. However, inkjet printed metallic electrodes may exhibit poor electrochemical response at solution/electrode interface due to additives in ink formulation. To solve this problem, we modified the inkjet printed electrodes by electrochemical deposition of gold and silver particles on the surface. First, an array of three-electrodes pattern was printed on a modified polyethylene terephthalate substrate by jetting silver ink using a piezoelectric inkjet printer. Then, solutions of 2 mM HAuCl4 in 0.5 M H2SO4 and 0.3 mM AgNO3 in 3 M NH4OH were used to deposit gold and silver on working and counter/reference electrodes, respectively, by applying -2 V potential against platinum wire using CHI 660E potentiostat. The surfaces of the modified electrodes were characterized by optical and electron microscopy. Electrochemical characterization was performed by voltammetric methods using 0.5 mM Fe(CN)6\(^{3-}/4^-\) solution in 10x phosphate-buffered saline (pH = 7.4). Fidelity of metal modified electrodes were found to be stable on substrate than unmodified printed electrodes which were stripping off from the substrate after single electrochemical measurement. Using differential pulse voltammetry, modified electrodes detected dopamine—a model analyte and an electroactive neurotransmitter—with limit of detection of 100 nM in phosphate buffer, while the unmodified electrodes were insensitive towards discriminating dopamine from blank. In conclusion, sensitivity of inkjet printed sensors was remarkably enhanced by electrochemical metal deposition, which can be used to mass produce inexpensive electrochemical sensors while maintaining their low cost.

Keywords: Bioanalytical, Biosensors, Electrochemistry, Electrodes
Application Code: Bioanalytical
Methodology Code: Chemical Methods
Mass Spectrometry has become a powerful tool in the field of proteomics. When paired with Liquid Chromatography, complex samples of proteins can be separated, analyzed, and identified with the help of a protein database. We used a High Performance Liquid Chromatograph (HPLC) and an Electrospray Ionization tandem Quadrupole Time-of-Flight Mass Spectrometer (ESI-QTOF MS/MS) to identify proteins in Tetrahymena thermophila, a useful model organism. Various aspects of the procedure were altered so we could compare the results and analyze the effectiveness of different methods for proteomics using LC-MS/MS. We employed various cleaning methods including dialysis, spin desalting columns, molecular weight cut-off filters, and C18 spin tubes to clean-up the protein samples. We used both dithiothreitol (DTT) and Tris(2-carboxyethyl)phosphine hydrochloride (TCPH) to see if one breaks down the disulfide bonds more effectively. Lastly, we experimented with the Barocycler pressure cycling instrument to find the optimal settings for digestion. Samples were run through the LC-MS/MS and proteins were identified by comparing the fragmentation patterns to a protein database using MASCOT. Examining the results from the different methods of sample preparation allowed us to analyze the effectiveness of each method for proteomics using LC-MS/MS.
Cloth fibers found at the scene of a crime can be used as circumstantial evidence in a court of law. Current methodology can only determine the color and chemical composition of the sample, giving limited information about its origins and often leaves fibers indistinguishable. Isotope ratio mass spectrometry (IRMS), however, can be used to analyze the ratio of carbon, hydrogen, nitrogen, and oxygen isotopes, further differentiating fibers of the same color and chemical composition. For the purpose of this study, synthetic fibers, including carpet fibers and rope, were analyzed using IRMS. Additionally, the effect of chemical (i.e. DNA fingerprinting chemicals) and environmental factors (i.e. sun, rain, snow, and aquatic environments) on the stable isotope ratios of natural and synthetic fibers were investigated to determine whether the forensic value of fiber analysis is compromised by chemical and environmental factors.

**Keywords:** Forensics, Identification, Isotope Ratio MS

**Application Code:** Homeland Security/Forensics

**Methodology Code:** Mass Spectrometry
This study aims to investigate the volatile organic compounds (VOCs) present in the human epidermis and the uniqueness and trends of VOCs among different races, ages, genders and environments. In this study, GC-MS was used to analyze the type and amount of VOCs. Approximately 50 VOCs were identified as common in skin samples, and some compounds might be markers for certain races and environments. Among those compounds, cyclohexane,1,3-dimethyl-,cis- might be a marker for Africans and Asians since it has only been found on African and Asian participants. Ethane,1,1-difluoro and propane were seldom found on white participants but often on other races; therefore, they might be markers for people of color. The amount of acetaldehyde might also be a marker for different races. Hispanics have the highest acetaldehyde level following by blacks, whites and Asians. In addition, acetonitrile was only found on St. Olaf students and faculty members; therefore, it might be an indicator for subjects who have spent considerable time at St. Olaf College.

Keywords: Forensics, Gas Chromatography/Mass Spectrometry, Identification
Application Code: Homeland Security/Forensics
Methodology Code: Gas Chromatography/Mass Spectrometry
An Improvised Explosive Device (IED) leaves minimal evidence after detonation which can make it difficult for those scenarios to be investigated. Currently, a precise method for determining the specific origin of an IED is not used. With Isotope Ratio Mass Spectrometry, only a very small fragment is necessary to acquire results, which makes it a useful method for the investigation of IEDs. Isotope Ratio Mass Spectrometry gives an extremely accurate measure of the relative abundance of isotopes in atoms such as Carbon, Nitrogen, Hydrogen, and Oxygen in a given sample. In this project, wire insulators of various colors and manufacturers are analyzed to determine if there is a statistical difference in their isotope ratios. If so, forensic scientists will be able to differentiate wires from one another and test IED residues to see if they match wire that was found in a suspect’s possession.

Keywords: Forensics, Isotope Ratio MS, Polymers & Plastics
Application Code: Homeland Security/Forensics
Methodology Code: Mass Spectrometry
Many people use candles every day without thinking about the chemicals that they inevitably inhale. This project aimed to determine the identities and concentrations of volatile organic compounds (VOCs) present in unscented, undyed candles. A previous project aimed to identify the VOCs present but only determined their relative abundances. In the current project, an internal standard containing four compounds was used each time samples were run in the gas chromatograph-mass spectrometer (GC-MS) so that concentrations rather than relative abundances could be determined. Five types of wax were examined: beeswax, paraffin, soy, gel, and palm oil. The fumes released from each burning candle were collected and analyzed eight times using GC-MS to determine the VOCs present. Based on data from the previous project, one VOC, benzene, was chosen to have its concentration determined. The concentration present in the gas was much lower than suggested control parameters for human exposure.

Keywords: Consumer Products, Gas Chromatography/Mass Spectrometry, Identification, Volatile Organic Comp
Application Code: General Interest
Methodology Code: Gas Chromatography/Mass Spectrometry
**Session Title**: Emission Measurements of Low Molecular Weight Compounds from Commercially-Used Polymeric Materials Induced by Heat and Sun-Light Treatment

**Primary Author**: Akihiro Yamasaki
**Co-Authors**: Miyuki Noguchi

**Abstract Text**

Emissions of low molecular weight compounds from commercial products made of polymeric materials under high temperature or artificial sun-light exposure were measured by the passive flux sampling method. The polymeric materials tested include PVC, PE, PP, EVAL, PMMA, PET, POM, and PC. The passive flux sampler was made of a glass petri-dish (30 mm diameter, 5.0 mm height). A known amount of Tenax GR was set on the dead-end side of the sampler for capturing the gaseous compounds diffused through the sampler. The sampler was attached on the surface of the sample materials for a given period. Tenax GR was taken out after measurement, and the captured compounds were analyzed with ATD-GC-MS technique. The temperature was changed in the range of 25 to 75[^oC]. For the artificial sun-light experiment, the polymeric material sample attached with the sampler was placed under the artificial sun-light simulator, which radiates artificial sun light on to the surface of the sample material. The results demonstrated that the higher temperature conditions resulted in the increase in the number of chemicals emitted as well as the emission rates. The emitted chemicals include monomer molecules that would be generated by the dissociation of the polymer chain, additive chemicals such as plasticizers, and solvents that might be used during the production processes. The obtained results might be useful for the evaluation of risk caused by the emission of toxic chemicals during the use of commercial products made of polymeric materials.

**Keywords**: GC-MS, Polymers & Plastics, Thermal Desorption, Volatile Organic Compounds

**Application Code**: Polymers and Plastics

**Methodology Code**: Gas Chromatography/Mass Spectrometry
A new measurement method of nicotine in indoor air was developed as a maker chemical of environmental tobacco smoke (ETS) and the third hand smoke (THS). The method was applied to the evaluation of the personal exposure to ETS and THS. The method is based on capturing nicotine both in the gaseous phase and in the form of aerosol with a glass filter as a capturing media. L-ascorbic acid was added into the glass filter so that the retention capacity of nicotine was significantly improved. After a given period of sampling of indoor air including ETS or THS, the glass filter was detached and the amount of nicotine captured was analyzed by ATD-GC/MS. Two types of air sampling methods were developed; the semi-active sampling method using a specially-designed sampling device, and the passive flux sampling method with a stainless-steel petri dish. The semi-active sampling method was applied to the personal exposure to ETS for a smoker and a passive smoker. The personal exposure to ETS of the passive smoker was found to be about 53.5% of that of the smoker. The passive flux sampling method was applied to the emission of nicotine from the clothes and ceiling of a residence room where smoked to evaluate the personal exposure to THS. The emission of nicotine from clothes of the passive smoker was 2.5 g m^{-2} h^{-1}, which is almost equal to that of the smoker (2.6 g m^{-2} h^{-1}). The emission rate of nicotine from the ceiling was 2.0 g m^{-2} h^{-1}.
Development of Portable Fluorescence Detection System Using an Organic Photodiode Array Detector

Abstract Text

Miniaturization of analytical instrument is important in on-site analysis. An inorganic photodiode array (IPDA) is typically used in a desktop size photodetector which enables simultaneous multipoint detection. However, in the case of portable analytical instrument, the use of IPDA is not suitable in terms of the cost. On the other hand, an organic photodiode array (OPDA) can be fabricated onto a glass or plastic plate by simple and inexpensive manufacturing process. In this study, a 9-channel OPDA was fabricated on the glass plate using photolithography and vacuum deposition method. Then a novel fluorescence detection system for ELISA was developed using 9 LEDs and the OPDA. The performance of the present fluorescence detection system was evaluated using a laboratory made 9-well PDMS microtiter plate and aqueous resorufin solutions. The calibration curve for resorufin was linear for concentrations under 7.8 M with a correlation coefficient of 0.996. The limit of detection (LOD) (3.3 M) was estimated to be 0.24 M, while that obtained by commercially available 96-well mictotiter plate and desktop size microplate reader was 0.55 M. These results show that the sensitivity of present system is comparable to that of desktop size analytical instrument. Furthermore, the present system was applied to ELISA for the determination of IgA, a marker for human stress. The sigmoidal calibration curve for IgA was obtained and then the LOD was estimated to be 3.4 ng/mL. The detection sensitivity of present system is sufficient to evaluate human stress since human saliva contains 110-220 ng/mL of IgA.

Keywords: Biosensors, Immunoassay
Application Code: Bioanalytical
Methodology Code: Sensors
In recent years, counterfeit pharmaceuticals have increased in both popularity and availability, with on-line sellers offering low prices, fast delivery, and discreet purchasing of medications without the need for a doctor’s prescription. In addition to significant revenue losses for pharmaceutical companies, these drug ‘fakes’ pose dangerous health risks to consumers, since their composition is varied and unregulated. Sildenafil citrate, a prescription medication used to treat erectile dysfunction, is one of the most commonly counterfeited drugs worldwide. This study employs direct analysis in real time—time of flight mass spectrometry (DART-TOFMS) combined with multivariate statistical analysis for the determination of sildenafil citrate tablet authenticity, and evaluates the potential of this methodology for distinguishing illicit manufacturing source.

The rapid ambient ionization associated with DART, combined with the inherent high mass accuracy of TOFMS, suggest the suitability of this technique for high throughput screening of pharmaceutical authenticity by eliminating the need for sample preparation and chromatographic separation. In this study, mass spectra were obtained for authentic sildenafil citrate tablets as well as 85 pills obtained from ten separate counterfeit drug seizures. In-source collision induced dissociation (CID) was performed at MS inlet voltages ranging from 20 to 120V, and experiments to optimize ionization temperature, ionization mode (positive vs. negative), manner of sample introduction (manual vs. automated), and sample formulation (powdered vs. dissolved vs. intact tablet) were conducted. The resulting DART-TOFMS chemical signature data were processed using multivariate statistical methods and evaluated to determine which conditions provide the most discriminating information in terms of authentication, differentiation, and potential for identifying manufacturing source.

Keywords: Mass Spectrometry, Method Development, Pharmaceutical, Statistical Data Analysis

Application Code: Pharmaceutical

Methodology Code: Mass Spectrometry
The use of direct analysis in real time—time of flight mass spectrometry (DART-TOFMS) allows for rapid throughput of large sample sets. The resulting data can be compared using multivariate statistical methods that allow for the study of inter-sample similarity and complex sample characterization. The efficacies of different modes of dataset compression were compared in the analysis and sample classification of authentic and counterfeit sildenafil citrate, a prescription medication used to treat erectile dysfunction. The overarching challenge of the study was to develop a means of analyzing the data that avoids both over-fitting the available data, given the relatively small data set, as well as under-fitting the data through the use of overly stringent pre-processing and dimensionality reduction steps.

The study aimed to couple a dimensionality reduction method with a classification method that would allow for both the discrimination between spectra obtained for counterfeit and authentic sildenafil citrate samples as well as for accurate classification of unknown samples. Classification of uncompressed and compressed data for unknown samples was compared using a number of different classification methods: Euclidean distance, z-score, one versus all logistic regression, and support vector machine learning. The Euclidean distance classification method was the most accurate for both principal component analysis (PCA) as well as linear discriminant analysis (LDA). The more rigorous classification algorithms, logistic regression and support vector machine, were more accurate for the uncompressed data set.

Keywords: Other Hyphenated Techniques, Pharmaceutical, Statistical Data Analysis, Time of Flight MS
Application Code: Pharmaceutical
Methodology Code: Data Analysis and Manipulation
We have used electrogenerated iodine coulometry for the determination of reducing agents such as ascorbic acid and selected sulfur compounds (thiosulfate and glutathione) in our instrumental analysis laboratory course. Our instrument is composed of a selectable constant current source connected to twin Pt electrodes contained in a beaker mounted on top of a photodiode sensor wired to a homemade current-to-voltage circuit. The darkening of the solution due to the formation of the blue iodine-starch complex is monitored by the photodiode and the circuit voltage output is recorded as a function of time. The overall output profile looks similar to a titration curve; the peak of the first derivative plot is considered to be the endpoint time.

To the best of our knowledge, the application of iodine coulometry for the determination of transition metals has not been reported. We have discovered that the coulometric titration of cysteine when complexed with Zn$^{2+}$ is slower as compared to uncomplexed cysteine. Using a constant [cysteine] at a minimum of a two-fold excess to the metal ion at pH 9.1, the endpoint time has been found to be proportional to [Zn$^{2+}$], ranging from 400 sec for only cysteine to 500 sec for cysteine in the presence of 6 x 10^{-5} M Zn$^{2+}$. The % RSD of the data in our calibration plot averages about 6%; presently we are controlling the temperature of the titration solution to try to minimize this RSD value. The determination of zinc in supplement tablets is underway.
A liquid sampling-atmospheric pressure glow discharge (LS-APGD) source for optical emission spectroscopy (OES) is described. The LS-APGD-OES microplasma method requires smaller sample volumes (10s of microliters) and less power (<50 W) than industry standard instrumentation such as ICP-OES. Additionally, the instrument runs in a total consumption mode, offering another advantage over current instruments in terms of potential field applications. Analytical characterization of the instrument included optical spectra assessment, electrolytic solution composition and flow rate, carrier gas flow rate, and inter-electrode separation distance. The electrolyte solution was found to be best at a concentration of 5% HNO3 and a solution flow rate of 45 uL/min. The flow rate of the solution has a large effect on the stability of the plasma and the broadening of resulting temporal profiles of injection transients. The sheath gas flow rate has less of an effect on analytical lines but contributes greatly to the stability of the plasma. Flow rates in excess of 0.600 L/min cause excessive spray of electrolytic solution while flow rates below 0.400 L/min melt the inner capillary and prevent plasma formation. The inter-electrode separation distance, the distance between the anode and cathode, is of great importance in experimentation. The distance must be kept to under 3 mm to ensure plasma stability and analyte peaks of sufficient intensity. Studies were performed with multiple single-element solutions as well as multi-element solutions. This instrumentation is compact and has potential applications for in-field analysis where practical alternatives are severely lacking.

Keywords: Nuclear Analytical Applications, Plasma Emission (ICP/MIP/DCP/etc.)
Application Code: Nuclear
Methodology Code: Atomic Spectroscopy/Elemental Analysis
The operating parameters for liquid sampling-atmospheric pressure glow discharge (LS-APGD) microplasma have been evaluated as an ambient desorption optical emission source for the purpose of elemental analysis. Optical (atomic) emission is being pursued due to its greater flexibility than mass spectrometry for field-based measurements. Use of the microplasma in an ambient desorption (AD) mode allows for direct solids analysis of diverse materials in their native state. Parameters that were evaluated include discharge current, electrolyte liquid flow rate, sheath gas flow rate, counter gas flow rate, and angle of liquid electrode. Furthermore, varying metals, electrolyte solution identity, and different matrix forms of copper substrate were evaluated in order to better understand the precise mechanisms of operation. The intensity of the atomic emission, standard deviation, and signal-to-noise ratios were analyzed to gather understanding of how the LS-APGD operates and how the volatilization of the metals occurs. The results indicate that the identity of acid has little effect on the volatilization of different metals, however the intensity of emission is still heavily dependent on the type of metal. Increases in electrolyte flow rate increased the intensity of emission (logarithmically) but also decreased the standard deviation of the emission signal. Other key parameters that determine the intensity of the signal were the counter gas flow rate and angle of incidence of the plasma onto the solid sample. It is believed that the LS-APGD operating in an AD-OES mode has a great deal of potential for field-deployment applications.

Keywords: Elemental Analysis, Forensic Chemistry, Nuclear Analytical Applications, Plasma Emission (ICP/MIP/D
Application Code: Nuclear
Methodology Code: Atomic Spectroscopy/Elemental Analysis
Studying of Hydrodynamic and Loading Characteristics in Analytical Protein Separations on Polypropylene Capillary-Channeled Polymer (C-CP) Phases

In the field of protein analytics, the coupling of liquid chromatography separations, especially reversed phase (RP), and electrospray ionization mass spectrometry (ESI-MS) provides a great deal of information. Capillary-channeled polymer (C-CP) fibers have been employed for fast protein separations on both the analytical and preparative scales, explicitly for the capability to operate at high linear velocities (>50 mm s⁻¹), without van Deemter C-term penalties. Because of the high hydrophobicity and salt matrix removal functionality, polypropylene (PP) has been used for protein separation through RP mode. In order to maintain the preferred high linear velocities, while also using volume flow rates compatible with standard ESI-MS, smaller column diameters (i.d. = 0.5 mm) are evaluated with the intension to achieve high chromatographic throughput. In order to study the hydrodynamic characteristics in C-CP fiber protein separations, different operational parameters required evaluation, including gradient rate (elution volume) and flow rate etc. Their effects on peak shape, resolution, and efficiency were correlated. In addition, volume and mass loading effects were studied on the C-CP fiber stationary phase as a function of pH variance using bovine serum albumin (BSA) as the model protein. For the ultimate LC-ESI-MS applications, mixtures of ribonuclease A, cytochrome c, myoglobin and lysozyme were prepared in phosphate buffered saline (PBS) and urine matrices. The efficiency of the matrix removal is reflected in the near-identical qualitative and quantitative responses. It is considered that C-CP fiber columns can provide the comparably high throughput and recoveries desired in top-down proteomics applications.

Keywords: HPLC, Liquid Chromatography/Mass Spectroscopy, Separation Sciences
Application Code: Bioanalytical
Methodology Code: Liquid Chromatography
Graphene quantum dots (GQDs) exhibit unique properties that have sparked interest in their use as alternative fluorescent probes in place of organic dyes and inorganic nanoparticles. GQDs are safe, economical, and easily synthesized water soluble nanomaterials with an emission maximum at 460 nm, independent of excitation wavelength from 300 nm – 420 nm. We have shown that enhancement in the fluorescence emission of GQDs upon binding to small molecule analytes, such as triacetone triperoxide, is concentration dependent. Such binding with GQDs occurs via noncovalent mechanisms and can be exploited for analysis of a range of other targets such as illicit drugs (bath salts) and ssDNA. Additionally, it should be possible to mediate the electrophoretic mobility of ssDNA targets by the introduction of GQDs to the separation buffer in capillary electrophoresis-based experiments. The use of GQDs in this capacity will yield the additional benefit of rendering the associated DNA fluorescent, thus facilitating its sensitive detection. Buffers to be employed in analyte mobility studies will incorporate GQDs synthesized in-house according to a bottom-up method from citric acid or other simple carbon precursors. Synthetic conditions will be modified as necessary to achieve reproducible and uniform dots with optimal spectral properties. Characteristics of the resulting GQDs will be assessed by spectroscopy (UV/Vis, luminescence, FTIR, and Raman) and microscopy (AFM and TEM). Initially, we will screen for changes in small molecule or DNA target mobility as a function of added GQD concentration. GQDs will serve these studies in two capacities: to improve resolution of bound and unbound small molecule or DNA sequences and to provide label-free fluorescence detection of bound targets.

Keywords: Capillary Electrophoresis, Characterization, Drugs, Fluorescence
Exposures in the area surrounding a manufacturing or chemical plant are regulated by the EPA Clean Air Act of 1990 and subsequent amendments. Since that time, more than 120 regulations have been issued. In Oct. 2015, the EPA issued a new limit of 2.8 ppb of benzene for fence lines at refineries. Fence line monitoring is important since fugitive emission sources account for most of the VOC inventory according to EPA.

Gas chromatography is one of the detection methods mentioned by EPA. The detector described by EPA is the flame ionization detector (FID). Since the FID does not detect low ppb levels of benzene, a thermal desorber is required. The photoionization detector (PID) is 50-100 times more sensitive than the FID and does not require any support gases like H2 or zero air. The PID can detect < 1 ppb of benzene by direct injection. We have also developed a low power concentrator that can provide a 100 fold concentration of an air sample that can be desorbed in < 3 seconds. We have developed a capillary column that can separate and detect benzene specifically in under 2 minutes in the presence of 50 fold excess of other refinery hydrocarbons.

The GC/PID will be compared with the GC/FID method for precision and accuracy of low and sub ppb levels of benzene and other VOC’s in refinery atmospheres.

Keywords: Environmental/Air, Gas Chromatography, Gasoline, Portable Instruments
Application Code: Environmental
Methodology Code: Gas Chromatography
In this study, a GC-MS technique was developed and applied to determine 17α-ethinylestradiol (EE2), an active ingredient of oral contraceptives, and its fate in Lake Quinsigamond, Massachusetts, USA. To the knowledge of the authors, this is the first study of EE2 and its microbial and photochemical degradation in a lake ecosystem. EE2 was detected at a concentration up to 11.1 ng L\(^{-1}\). At this concentration EE2 may affect the reproduction of fish and other aquatic organisms in the lake due to its high estrogenic activity. EE2 was persistent to the biodegradation by the microorganisms in the lake. Under aerobic conditions a long lag phase (42 days) was observed before the biodegradation of EE2 and a half-life of 108 days was estimated. Under anaerobic conditions, EE2 experienced even a longer acclimation stage (63 days) and a slower microbial degradation in the lake water. The photodegradation of EE2 was rapid in the lake surface water under natural sunlight, with a half-life of less than 2 days in summer sunny days. Compared to biodegradation, photodegradation may represent a predominant removal mechanism for EE2 in natural surface waters.
In this study, green chemistry principles have been integrated into analytical chemistry curriculum. Two environmentally friendly HPLC methods, a reversed-phase (RP) HPLC method and a hydrophilic interaction liquid chromatographic (HILIC) method, were developed in the analytical teaching laboratory for the determination of renal function biomarkers, creatinine and uric acid, in human fluids. In the RP-HPLC method, the separation was achieved by using a RP C18 column with an aqueous mobile phase of phosphate buffer. The retention loss of a C18 column caused by the highly aqueous mobile phases was avoided by employing a gradient elution using a small volume (< 0.23 mL) of acetonitrile and phosphate buffer at pH 4.75. Quantitation was carried out by internal standard method. The detection limits for creatinine and uric acid were 0.045 and 0.062 µg mL−1, respectively. In the HILIC method, a weak polar cyano HILIC stationary phase with a highly aqueous mobile phase was chosen for the separation to minimize the consumption of harmful organic solvents. Quantitation was also carried out by internal standard method. This developed isocratic HILIC method consumed less than 0.25 mL of acetonitrile per sample separation. The detection limits for creatinine and uric acid were 0.04 and 0.06 µg mL−1, respectively. Both of the methods provide a simple, rapid, accurate and sensitive detection for the species of interest, and have been successfully applied for the determination of creatinine and uric acid in human urine and tear samples.

Keywords: Analysis, Bioanalytical, Chromatography, Clinical Chemistry
Application Code: Clinical/Toxicology
Methodology Code: Liquid Chromatography
DNA microarrays allow rapid, multiplexed screening for specific DNA sequences related to disease states. The information density of standard microarray technology is limited by the $10^{-3}$ m scale of the capture areas or addresses on the arrays. Our goal is to scale DNA capture addresses down to individual molecules, reducing the address size to the nanometer (molecular) scale with the potential capability of detecting individual target oligonucleotides without amplification. As a step toward that goal, single-molecule microarrays were constructed by immobilizing recognition-probe ssDNA at random locations on a glass substrate using complementary-hybridization between a poly-adenine “anchor” sequence on the probe strand and poly-thymine ssDNA immobilized on the glass surface. These randomly-arrayed probe sites can be located to better-than 50 nm resolution by observing multiple (>25) individual reversible hybridization events with a complementary fluorescently-labeled target ssDNA. Near the melting temperature, DNA hybridization is reversible and the kinetics of duplex dissociation depend sensitively on the DNA sequence. This allows discrimination between two 10-mer recognition sequences differing by only one base-pair interaction based on a 4-fold difference in dissociation rate. The addition of a single nucleotide polymorphism (SNP) results in a significant change in the dissociation kinetics; mismatched DNA dissociation rates are up to 20 times faster than that of the fully-complementary sequence, allowing mismatched ssDNA to be identified and quantified. Furthermore, mixtures of fully-complementary and single-base-mismatched ssDNA in solution can be assayed by interpreting the distribution of fast and slow dissociation kinetics from probe molecules. This single-base pair sensitivity could be used to identify instances of single-nucleotide polymorphisms, oxidative damage, or possibly epigenetic modification of individual DNA strands.

**Keywords:** Fluorescence, Microspectroscopy, Nucleic Acids, Single Molecule

**Application Code:** Bioanalytical

**Methodology Code:** Biospectroscopy
### Abstract

Glycomics is the systematic study of all carbohydrate (glycan) structures in biological systems. An important subset of it is Analytical Glycomics that is widely applied recently in biomedical research and biopharmaceutical development.

Comprehensive glycosylation analysis offers a new avenue in biomarker discovery as changes in the carbohydrate moieties of glycoproteins can be sensitive indicators of the actual state of the underlying biochemical phenomena. High performance glycoanalytical methods are also needed in the biopharmaceutical industry, because of the oligosaccharide structures on therapeutic proteins play significant roles in their bioactivity and cytotoxicity. In this presentation the advantages of various glycoanalytical platforms will be conferred for comprehensive N-glycan analysis, mostly focusing on capillary electrophoresis and its hyphenation with mass spectrometry.

**Keywords:** Bioanalytical, Electrophoresis, Mass Spectrometry

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

**Methodology Code:** Capillary Electrophoresis
Today, the coupling of column separations (LC and CE) to mass spectrometry is widely used with applications in many areas. In the field of biotechnology, LC-MS and more recently CE-MS have become critical tools for many of the steps in bringing a biotherapeutic to market, from research to development to lot release. In this talk, we will explore several recent examples of the use of these tools in the development process. Specifically, we will describe separation-MS approaches to deep characterization of biotherapeutic products, examining intact and large fragments of the protein drug. The information obtained is shown to be complementary to peptide mapping. Further, new approaches for identification and quantitation of host cell protein impurities in the drug product using targeted and untargeted data independent acquisition will be described. As a final example, we will present the application of omics methods using LC-MS to bioreactors to provide biological insight into biopharmaceutical production. The examples will demonstrate the essential role of separation-MS in the many stages of the protein drug production process. To conclude the talk, we will suggest new directions of the biotechnology field that separation-MS will play a decisive role.
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**Abstract Text**

The modern monolithic columns emerged 25 years ago. While the early polymer-based monoliths were used in columns for the rapid separations of proteins and other large molecules, current literature describes a number of different applications in addition to typical chromatography demonstrating versatility of the monoliths. Several new formats have emerged recently that extend the use of monoliths in areas different from column chromatography. For example, polymer-based monolithic columns prepared using novel approaches enable efficient and rapid separation of small molecules. New chemistries have also been developed to afford monolithic columns for the separation in various modes and to control their selectivity. Modification of pore surface with nanoparticles and metal-organic frameworks is a current trend that extends applications of monoliths in the arena of highly selective fishing out systems. Thin monolithic layers are also gaining more attention since they allow efficient separations of proteins combined with mass spectrometry using very simple means. Monoliths also serve as supports for immobilization of enzymes to form very active enzymatic reactors.

**Keywords:** Analysis, Chromatography, Material Science

**Application Code:** Bioanalytical

**Methodology Code:** Liquid Chromatography
Shortly after miniaturized separations were introduced, the laser-based detectors stimulated considerable interest in the analytical chemistry community. Tightly focused laser beams at once became the ideal light sources to probe extremely small peak volumes associated with capillary LC and CE. From all suggested laser-based techniques, some featuring very sophisticated instrumental designs, the laser-induced fluorescence (LIF) became most popular and practical to be commercialized. The initial disadvantage of LIF detection was its lack of practical applications: besides a few instances where natural substances attune to the excitation wavelengths of readily available lasers, most molecules of interest do not contain a suitable fluorophore. Intensive search for fluorogenic and fluorescent tagging reagents represented the next chapter in LIF detection. Perhaps the most illustrative case was carbohydrates, which can be uniformly tagged at their reducing end with a fluorescent reagent (which also contains charged groups suitable for electromigration). From a number of LIF-detection approaches, the method proposed by Guttman and coworkers in 1996, using the APTS reagent, has become most applicable in the analysis of complex carbohydrate mixtures. The emerging interest in glycobiology has fortunately coincided with the development of CE-LIF. This methodology has now been successfully applied to complex mixtures of polysaccharides, glycans released from complex glycoprotein mixtures as well as recombinant pharmaceuticals. In our recently published studies, CE-LIF in a microchip format has been utilized in the cancer biomarker investigations. With its resolving power and the capability of displaying isomeric glycans, CE-LIF is complementary to mass spectrometry in important biomedical applications.

Keywords: Bioanalytical, Biomedical, Capillary Electrophoresis, Carbohydrates
Application Code: Bioanalytical
Methodology Code: Capillary Electrophoresis
Abstract Text

We have developed and applied methods to annotate the architecture and dynamics of protein complex networks by utilizing a novel whole-network affinity pull-down method coupled to novel computational analysis tools. This method involves generation of TAP-tagged node proteins, which are expressed, pooled and affinity-purified in parallel. This approach allows for efficient system-wide identification and quantification of protein complex members and novel interactors in response to perturbation in a highly efficient manner. This proteomic platform has been enabled through development and application of biochemical and proteomic methodologies to study network response to known perturbations in established protein networks. Specifically, as a proof-of-principle, the developed platform has been employed to study changes in nutrient sensing protein complexes in response to rapamycin treatment in Saccharomyces cerevisiae.

This concept has been extended to the DNA damage response as well as energy homeostasis networks in yeast, and the latter correlated to a homologous mammalian network for investigation of metabolic dysregulation in disease. By measuring the dynamics of protein networks, we have identified novel associations within and between components that have otherwise been missed in traditional protein-by-protein Affinity Purification-Mass Spectrometry (AP-MS) methodologies, and this is the first proteomic platform to enable dynamic interaction measurements at a network-wide scale.

Keywords: HPLC, Mass Spectrometry, Proteomics, Tandem Mass Spec
Application Code: Bioanalytical
Methodology Code: Mass Spectrometry
The Pittsburgh Conference Achievement Award (Dauenhauer)

Universal Carbon Detector (UCD) for Calibration-Free Quantification of Complex Mixtures

Quantification of chemical mixtures is the basis for modern chemistry and chemical process applications related to food, energy, chemicals, pharmaceuticals and agricultural technologies. Mixtures of organic chemicals can comprise hundreds of compounds, necessitating expensive and time-consuming separation and detection with conventional calibration techniques. In this work, we introduce a catalytic microreactor which allows for calibration-free quantification of organic compounds within gas chromatography[1,2]. Analyte compounds eluting a conventional gas chromatograph column flow into the microreactor, where a series of catalytic reactions convert each analyte to methane (>99.9%). Subsequent detection via flame ionization thus results in a common carbon response factor for all compounds. By this approach, mixtures of hundreds of compounds including sugars, aldehydes, organic acids, alcohols, thiophenes, and many other hydrocarbons can be quantified without calibration. Implementation of the Universal Carbon Detector (UCD) [3] simplifies chemical research and introduces new capability for analyzing mixtures. Elimination of calibration permits rapid analysis of large complex mixtures that exist in applications such as fuels. In particular, biofuel mixtures comprised of hundreds of highly oxygenated organic compounds benefit from the UCD; quantification (i.e. determination of carbon %) is greatly simplified. Additionally, the UCD permits the quantification of molecules such as carbon dioxide, which were not previously measurable without a second detector. Another benefit of UCD is the capability for quantifying compounds such as formaldehyde or formic acid, which exhibit low response in an FID – the UCD method increases the limits of detection of these compounds by one-to-two orders of magnitude.


Abstract Text

Co-Authors
Abstract Text

Specifying the analytical techniques and methods can be very challenging when considering a large number of sample streams to be analyzed and high analysis frequency. Combining those constraints with the need to monitor many components over a wide range of concentrations makes at-line analytical approaches preferred by many analytical scientists. The recent introduction of a commercial universal carbon detector that can be combined with a gas chromatograph with flame ionization detection, has provided the ability of quantifying components without the need of running a full calibration, and even when the reference material is not present or when only knowing the molecular formula of unknowns. In this presentation we will describe an at-line implementation that combines automated sample preparation and gas chromatography with flame ionization detector and universal carbon response to provide flexibility and accuracy with minimal information of unknowns or when reference materials are not available.

Keywords: Automation, Chromatography, GC Detectors, Volatile Organic Compounds
Application Code: Process Analytical Chemistry
Methodology Code: Gas Chromatography
This presentation shows the applicability of the Wasson-ECE TOTM reactor as a novel tool for total hydrocarbon analyses in a variety of fields. Current total hydrocarbon analyzers depend on the response to molecular weight relationship of the flame ionization detector. While valid for saturated aliphatic hydrocarbons, unsaturated and substituted hydrocarbon species produce errors with the quantitation using this technique. By conversion of all of the hydrocarbon components to methane, a more accurate analysis of total hydrocarbon content is possible using a uniform response factor for all components. This analysis has been proven useful in the analysis of high-purity gases, medical gases, air monitoring, and impurities in monomers. Example chromatograms and quantitative data will be presented.

**Keywords:** Detector, Gas Chromatography, GC Detectors, Hydrocarbons

**Application Code:** General Interest

**Methodology Code:** Gas Chromatography
The Expanded Toolbox of Universality of Non-Mass Spectrometric Detection for Gas Chromatography

Mass spectrometry (MS) has long been the detector of choice for laboratories measuring analytes found in complex mixtures, diverse chemical classes, and as a safeguard offering flexibility in future projects. While MS has proven to be quite successful for applications, instances arise where inherent MS pitfalls are encountered. Three recent non-MS detectors or in-line processes will be discussed in the context of increased compound detection, novel quantitative measures, and even qualitative spectral information. The GC-Tracera (Shimadzu Scientific Instruments) utilizes barrier discharge ionization from a He plasma to detect all GC-amenable compounds while increasing sensitivity compared to TCD or FID. For systems already making measurements with FID, the introduction of a Polyarc microreactor (Activated Research Company) expands the detection to all carbon-containing GC-amenable compounds through a 2-stage combustion/reduction process to produce methane from each carbon atom. Since methane is the analyte now measured, there are no response factors to consider in quantification, and values can be determined based upon a ratio with an internal standard. The VGA-101 (VUV Analytics) is a spectroscopic detector measuring analyte absorbance from 120 – 430 nm, found to universally detect and quantify gases and organic compounds based off Beer’s Law. Acquired spectra is also unique for compounds, allowing for coelution deconvolution and isomer speciation. These recent innovations provide additional options for laboratories and their capabilities as they expand their research or quantification confidence.

Keywords: Data Analysis, Gas Chromatography, GC Detectors, Instrumentation

Application Code: General Interest

Methodology Code: Gas Chromatography
GC and GC/MS are fantastic analytical tools. I was fortunate to be an interested observer and participant in the early stages, and then later at Virginia Tech, able to use them in a variety of interesting applications; teaching Chromatography at the FBI Academy, Quantico, VA for 12 years, leading to serving on a National Academy of Science Committee "Airport Security" just prior to Desert Storm, and later nicely funded by FAA, TSA and final Homeland Security to:

1. Train dogs to smell bombs
2. Develop TAs (training aids, non-energetic for training dogs)
3. Develop GC/ECD and GC/MS methods and libraries for all terrorist bombs for which residues were made available
4. Solving the mystery of the Atlanta Olympic Bombing (not a terrorist attack) and characterizing all of Col. Quadaffi Semtex Bombings

Keywords: Forensics, GC, GC-MS
Application Code: Homeland Security/Forensics
Methodology Code: Gas Chromatography/Mass Spectrometry
Enhanced sample multiplexing is very helpful for increasing experimental throughput and answering questions about protein changes across a number of sample conditions. Experimental designs that include longitudinal timepoints, multiple tissues, disease stages and types, and large clinical cohorts can benefit tremendously from the ability to analyze many samples in a single analysis. Our group has developed a quantitative proteomics approach termed cPILOT, which combines precursor isotopic labeling with isobaric tagging. The advantages of the cPILOT approach include flexibility to combine various precursor labels with reagents such as TMT-10 plex to tag more than 20 samples in a single analysis. Additionally, cPILOT is versatile and can be modified to measure post-translational modifications. This presentation will discuss our recent efforts to improve the performance of cPILOT, further enhance multiplexing capability, and add to the versatility of cPILOT for post-translational modification analysis. Additionally, the application of cPILOT to study changes in aging and disease will be presented.

Keywords: Bioanalytical, Mass Spectrometry, Proteomics
Application Code: Bioanalytical
Methodology Code: Liquid Chromatography/Mass Spectrometry
Matrix-assisted ionization (MAI) mass spectrometry (MS) spontaneously transfers compounds, at least as large as the 66 kDa bovine serum albumin protein, into gas-phase ions when the matrix:analyte sample is simply exposed to sub-atmospheric pressure conditions of a commercially available mass spectrometer source. This new ionization process can be assessed from atmospheric pressure at the inlet of a mass spectrometer or by introduction of the sample directly into vacuum using, for example, an intermediate pressure MALDI source. Gas-phase ions are observed multiply protonated even using aprotic matrices such as 3-nitrobenzonitrile and 1,2-dicyanobenzene. Over 40 additional MAI matrices have been discovered and all have in common that charge separation occurs when ejected into a tube connecting a higher to a lower pressure region. While there is no specific structural motif, functionalities leading to less volatility and charge competition, e.g., carboxylic acids, typical for MALDI matrices, are not present. Instead, all MAI matrices have in common that they sublime in vacuum, and thus are no more likely to contaminate the mass spectrometer ion optics than solvents used with ESI or APCI. The method has excellent sensitivity having a limit of detection of <50 attomoles, depending on the mass spectrometer used, and producing clean full-scan mass spectra consuming only a few femtomoles of, for example, drugs and peptides. Complex mixtures can be acquired in seconds directly from buffered solutions, biological fluids and tissue. By placing matrix only on the feature of interest on a surface and exposing the surface to the vacuum of the mass spectrometer, ions are observed only from compounds exposed to the matrix solution allowing rapid interrogation of ‘features of interest’.

Keywords: Biological Samples, Mass Spectrometry
Application Code: Biomedical
Methodology Code: Mass Spectrometry
One of the most challenging problems in biochemistry involves understanding how proteins fold. After more than 50 years of work, experimental characterization of protein folding usually leads to results which are described as a cooperative, two phase, transition between the folded and unfolded states – i.e., the protein appears to melt. Here we present new data from an IMS-MS analysis of simple proteins that are electrosprayed from a temperature controlled source. The results suggest that the cooperative two state behavior involves other states that are captured in the IMS-MS analysis. In some examples, we find evidence for at least 10 structures that arise at slightly different transition temperatures. The ability to experimentally capture information about new states that are involved in folding and unfolding events may help guide theoretical efforts to model folding processes.
While cutaneous melanoma represents just 4% of skin cancers, it accounts for ~80% of skin cancer-related deaths. Extensive genomic analysis has revealed several subtypes characterized by gene mutations and includes the BRAF subtype which makes up 52% of all melanomas. However, knowledge on the molecular spectra of tumors from underrepresented minorities is limited and it remains unknown whether targeted approaches are clinically useful in such populations. This study sought to compare the melanoma mutational spectra in Caucasians, Latinos, and African Americans. Using the Cancer Genome Atlas Project, clinical and genomic data was downloaded from 470 patients diagnosed with melanoma and analyzed for the most frequently mutated genes, stratified by Race and Ethnicity. The data set included 342 Caucasians (96.3%), 1 African American (0.3%), and 5 Latinos (1.4%). BRAF was the most commonly mutated gene with 319 patients presenting with a BRAF mutation (66.7%). Mutations V600E (27.0%) and V600K (4.2%) were the most frequently found BRAF mutations. Caucasians had a large number of BRAF mutations (53.8%) with V600E and V600K making up 73.9% and 9.9% of the BRAF mutations, respectively. Additional mutations were found in many other genes. Of the Latinos, 2 had the V600E/K mutations. The one African American patient had a mutation in APOB. While the data was limited by the small number of minorities, our data demonstrates that BRAF remains a major player in melanoma. Knowing an individual tumor’s distinct molecular profile may help clinicians improve diagnosis and treatment, as targeted treatments are available for BRAF- and other mutations.
Proteomics can look at thousands of proteins simultaneously, making it an ideal platform for the investigation of drug targets. Here we describe differential intensity screening and ranking of unknown protein targets (DISRUPT); a method that ranks hundreds of thousands of high-resolution mass spectrometry features by statistical significance of drug stabilization. DISRUPT provides a novel, unbiased platform for ranking of unknown drug-protein interactions that does not require a priori information of the binding target.
Single cell analysis is required to understand cellular heterogeneity in biological systems. We propose that single cells (blastomeres) isolated from early stage invertebrate, amphibian, or fish embryos are ideal model systems for the development of technologies for single cell analysis. For these embryos, although cell cleavage is not exactly symmetric, the content per blastomere decreases roughly by half with each cell division, creating a geometric progression in cellular content. This progression forms a ladder of single-cell targets for the development of successively higher sensitivity instruments. In this manuscript, we performed bottom-up proteomics on single blastomeres isolated by microdissection from 2-, 4-, 8-, 16-, 32- and 50-cell Xenopus laevis (African clawed frog) embryos. Over 1,400 protein groups were identified in single-run reversed-phase liquid chromatography-electrospray ionization-tandem mass spectrometry from single blastomeres isolated from a 16-cell embryo. When the mass of yolk-free proteins in single blastomeres decreased from ~0.8 µg (16-cell embryo) to ~0.2 µg (50-cell embryo), the number of protein group identifications declined from 1,466 to 644. Around 800 protein groups were quantified across four blastomeres isolated from a 16-cell embryo. By comparing the protein expression among different blastomeres, we observed that the blastomere-to-blastomere heterogeneity in 8-, 16-, 32- and 50-cell embryos increases with development stage, presumably due to cellular differentiation. These results suggest that comprehensive quantitative proteomics on single blastomeres isolated from these early stage embryos can provide valuable insights into cellular differentiation and organ development.
Acute Myeloid Leukemia (AML) is the most common leukemia in adults. Despite the approval of 18 drugs for its treatment, AML has the lowest survival rate among leukemias. This mostly stems from the lack of a complete picture of the cellular events, as progression of AML affects multiple biochemical pathways including kinase signal transduction and chromatin/epigenetics. Due to its complex nature the scientific community has investigated AML using large-scale -omics strategies. This includes genomics studies using next-generation sequencing for DNA mutation analysis of blood and bone marrow cells; metabolomics of the blood using both mass spectrometry (MS) for lipid analysis and combined transcriptomics and proteomics using microarray technology and MS, respectively. All techniques provided a long list of promising results, but these have not been fully exploited, mostly due to the complexity of interpreting large-scale data. In general, the comprehensive list of changes in relative abundances of the analyzed molecules makes it difficult to define the direct cause-effect links, and which events are possibly just secondary effects due to system perturbation. We aim to investigate protein and phosphorylation dynamics of chromatin associated proteins upon FLT3 inhibition using quizartinib and, in parallel, analyze histone post-translational modifications (PTMs) cross-talk using our state-of-the-art MS based platform (i.e. to detect combinatorial PTMs occurring simultaneously on intact histone N-terminal tails). Our preliminary results demonstrate that we have the approaches and expertise to analyze protein PTMs from AML cells and investigate post-translational regulation of histone modifying enzymes established by dysregulated phosphorylation signal transduction cascades. Our aims will lead to the reconstruction of a system-wide network regulating AML response to treatment, which might reveal novel targets to increase its efficacy.
Protein glycosylation is common but a complicated form of posttranslational modifications of proteins. It is the only modification that has variable and intricate structures. Glycans play key roles in diseases and in maintaining health. Despite their importance, our abilities to measure and determine precise structures have been limited. Site-specific glycosylation is the most challenging particularly from the point of view of glycan heterogeneity. In this presentation, we illustrate the methods for site-specific analysis of glycoproteins with extensive heterogeneity for cell membrane proteins. We examine the role glycosylation plates in cellular membrane functions. Our research has focused on developing advanced separation methods, accurate and structurally informative mass spectrometry techniques and automated informatics tools that now make rapid throughput profiling of glycans possible. These methods are employed to determine the role of glycans in diseases allowing us to observe and characterize the glycan signature of cancer and autoimmune diseases in tissues and in blood. The cell membrane is composed of a glycan rich environment that mediates cellular interactions. Glycan profiling of cell membrane shows glycan changes during cellular transformation as cancer cells become differentiated and more metastatic. Glycan profiling can also elucidate the changes in glycosylation as host cells interact with microbes.

Keywords: Liquid Chromatography, Mass Spectrometry, Tandem Mass Spec, Time of Flight MS
Application Code: Biomedical
Methodology Code: Mass Spectrometry
Glycoproteins on the cell surface are ubiquitous and essential for human cells to interact with the extracellular matrix, communicate with other cells, and respond to environmental cues. Although surface glycoproteins can dramatically impact cell properties and represent different cellular statuses, global and site-specific analysis of glycoproteins only on the cell surface is extraordinarily challenging. We have developed effective mass spectrometry-based methods to specifically analyze surface glycoproteins. Surface glycoproteins metabolically labeled with a functional group were specifically tagged through click chemistry, which is ideal because it is quick, specific and occurs under physiological conditions. Sequentially, tagged glycoproteins were enriched for site-specific identification by mass spectrometry. Systematic and quantitative analysis of the surface N-glycoproteome in cancer cells with distinctive invasiveness demonstrated many N-glycoproteins up-regulated in highly invasive cells, the majority of which contained cell adhesion-related domains. Cell surface glycoprotein dynamics was also investigated and their half-lives were measured. The experimental results clearly demonstrated that surface glycoproteins with catalytic activities were more stable than those with binding and receptor activities. Glycosylation sites located outside of any domain had a notably longer median half-life than those within domains, which strongly suggests that glycans within domains regulate protein interactions with other molecules while those outside of domains majorly play a role in protecting protein from degradation. Considering the importance of surface glycoproteins, the newly developed methods will have extensive applications in the biological and biomedical research communities.

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

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

**Methodology Code:** Mass Spectrometry
Protein glycosylation has long been known to play fundamental roles in many biological processes, as well as in the pathological progression of many diseases. Glycoproteins also constitute the major biochemical class of current therapeutic targets and clinical biomarkers. However, protein glycosylation is the most structurally complicated and diverse type of protein modification and it poses great challenges for structural and functional studies.

We recently developed a novel chemoenzymatic method for the comprehensive characterization of glycoproteins. The glycans and glycosite-containing peptides were isolated and analyzed by mass spectrometry (MS), and the identified glycan and glycosite information was used to identify the intact glycopeptides for site-specific glycosylation analysis of glycoproteins using our recently developed software tools GPQuest by selection of N- and O-linked glycan-containing peptides and spectral library matching.

When applied the methods and software tools for the analysis of a standard glycoproteins or glycoproteins from cells, NGAG method allowed us to quantitatively analyze glycoproteins, glycans, glycosite-containing peptides (glycosites), and intact glycopeptides. This method provides a foundation for the large-scale and comprehensive characterization of glycoproteins.

Keywords: Carbohydrates, Informatics, Liquid Chromatography/Mass Spectroscopy, Sample & Data Management
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 in molecular engineering, nanomedicine and molecular elucidation of cancer biomarkers and theranostics.
Technologies capable of characterizing the full breadth of cellular systems need to be able to measure millions of proteins, isoforms, and complexes simultaneously. We describe an approach that fulfills this criterion: Adaptive Dynamic Artificial Poly-ligand Targeting (ADAPT). ADAPT employs an enriched library of single-stranded oligodeoxynucleotides (ssODNs) to profile complex biological samples, thus achieving an unprecedented coverage of system-wide, native biomolecules. We used ADAPT as a highly specific profiling tool that distinguishes women with or without breast cancer based on circulating exosomes in their blood. To develop ADAPT, we enriched a library of ~10\(^{11}\) ssODNs for those associating with exosomes from breast cancer patients or controls. The resulting 10\(^{6}\) enriched ssODNs were then profiled against plasma from independent groups of healthy and breast cancer-positive women. ssODN-mediated affinity purification and mass spectrometry identified low-abundance exosome-associated proteins and protein complexes, some with known significance in both normal homeostasis and disease. Sequencing of the recovered ssODNs provided quantitative measures that were used to build highly accurate multi-analyte signatures for patient classification. Probing plasma from 500 subjects with a smaller subset of 2000 resynthesized ssODNs stratified healthy, breast biopsy-negative, and -positive women.
Clinical evaluation of our factor IXa aptamer in two thousand patients undergoing angioplasty has demonstrated that aptamers can rapidly and potently inhibit their target proteins in patients and that antidote molecules can rapidly reverse such activity in the minute time frame. These observations suggest that aptamers may represent very useful molecules to control or monitor biochemical processes and blood coagulation in humans in real time. To begin to explore this potential further, we have started to evaluate the utility of antidote-mediated control of aptamers for a variety of other therapeutic and diagnostic applications. We will describe our recent progress developing a rapidly controllable factor Xa anticoagulant aptamer to effectively and reversible control blood coagulation during cardiopulmonary bypass surgery and a rapidly reversible thrombin aptamer for imaging blood clots in real time. Collectively these clinical and preclinical studies lead us to believe that rapidly controllable aptamers represent valuable therapeutic and diagnostic agents to monitor and control blood coagulation and thrombosis.
Advances in Nucleic Acid Ligand Screening Methods Against Extra-Cellular Targets

GlycoDNA Aptamers for HIV Vaccine Discovery

Currently there is no effective HIV vaccine, although some HIV patients spontaneously generate broadly-neutralizing antibodies (bnAbs). A promising approach is to develop structures which bind to the bnAbs and may mimic the native HIV structures bound by bnAbs. Toward that end, we have developed SELMA (SELection with Modified Aptamers), an aptamer method that enables the selection of carbohydrate-decorated DNA aptamers. Our glyco-aptamers bind tightly to bnAb 2G12 and are of interest as HIV vaccine candidates.

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Abstract Text

Currently there is no effective HIV vaccine, although some HIV patients spontaneously generate broadly-neutralizing antibodies (bnAbs). A promising approach is to develop structures which bind to the bnAbs and may mimic the native HIV structures bound by bnAbs. Toward that end, we have developed SELMA (SELection with Modified Aptamers), an aptamer method that enables the selection of carbohydrate-decorated DNA aptamers. Our glyco-aptamers bind tightly to bnAb 2G12 and are of interest as HIV vaccine candidates.

Keywords: Carbohydrates, Combinatorial Chemistry, Nucleic Acids, Pharmaceutical

Application Code: Drug Discovery

Methodology Code: Chemical Methods
A challenge for CTC-based diagnostic tests has been the development of methods with sufficient sensitivity to detect low levels of CTCs. Expense, accuracy and complexity have also limited clinical uptake of CTCs. To overcome these limitations we explored detecting CTCs by measuring their nuclease activity with nuclease-activated probes. We present the development of a rapid and highly-sensitive CTC detection assay based on nucleic acid probes that are selectively digested (activated) by target nucleases expressed in breast cancer cells. Nuclease activity in samples from women with Stage IV breast cancer and healthy donors was determined and correlated with clinical data. Blood samples were processed using microfilter units for CTC enrichment and converted into cell lysates that were examined by means of the probes. CTC-derived nuclease activity was quantified using a fluorometer. The presence of CTCs was confirmed using established CTC detection methods (e.g. immunohistostaining). The final study cohort included 28 breast cancer patients and 10 healthy donors. The averaged signal intensities from patient samples were significantly higher compared to the healthy donor control group, presumably arising from CTCs in the blood. The probe being the best predictor of disease yielded 100% sensitivity in the patient samples with a specificity of 70%. We describe a novel diagnostic for the detection of CTCs that could overcome limitations of current assays and could provide a robust diagnostic tool for breast cancer. Future clinical assays derived from this technology could require minimal training and infrastructure and might be developed into a point-of-care testing format.
Infectious diseases are one of the major reasons of deaths worldwide. Successful treatment of infection relies on a timely identification of the pathogen and its antibiotic resistance pattern in order to select the appropriate antibiotic treatment as early as possible. Classical microbiological analysis methods rely on time-consuming overnight cultures. Reliable culture-independent analysis methods could thus lead to huge improvements and help save lives by an early and targeted disease management. Raman spectroscopy plays a key role in turning this ambitious vision into reality because it allows for a fast molecular analysis of single microorganisms. Here, we will present our recent work towards the application of Raman spectroscopy as point-of-care test for an identification of pathogens on a single cell level in less than three hours. Before Raman spectroscopy can be used to identify pathogens, they have to be isolated from the sample matrix (e.g. blood, urine, sputum, ascitic). We will present chip-based bacterial isolation strategies (like e.g. a dielectrophoresis Raman setup) for a fast Raman spectroscopic identification of pathogens. Furthermore, it will be shown that not only the identification was realized but also the characterization of bacteria – drug interaction as a first step towards antibiotic susceptibility testing. Changes in the bacterial Raman-spectra due to antibiotic treatment can be identified already after 30 minutes of treatment. All this has been achieved by developing an automated Raman setup for use in clinics (BioParticle Explorer) together with innovative pathogen isolation strategies, which holds great promise as point-of-care-approach for diagnosis and therapy of infectious diseases.

Keywords: Biomedical, Chemometrics, Lab-on-a-Chip/Microfluidics, Raman Spectroscopy
Application Code: Biomedical
Methodology Code: Vibrational Spectroscopy
Clinical Biophotonics

New Advances in Molecular Spectroscopic Imaging

Current medical imaging tools rely on physical or physiological properties, rather than molecular content of the tissue. Without biomarker information, it remains difficult to differentiate metastatic diseases from the benign forms that can be left without treatment. For chemical content analysis, current strategy relies on tissue homogenization and separation, followed by various in vitro assays. This approach reveals the presence of molecules and their cellular concentrations. However, without spatial and temporal dynamics information, how molecules exactly execute their functions in a living system remains unknown. My research team is devoted to changing this conventional paradigm of medical imaging and bio-analysis through developing label-free molecular spectroscopic imaging technologies. In this presentation, I will present our latest advances in the molecular spectroscopic imaging field in both technology development (e.g. mid-infrared photothermal microscopy) and clinical translation (e.g. pump-probe imaging of glycated hemoglobin in single red blood cells).

Abstract Text

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Co-Author(s)

Bioanalytical, Biospectroscopy, Vibrational Spectroscopy
Biomedical
Biospectroscopy

Session Title
New Advances in Molecular Spectroscopic Imaging

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Bioanalytical, Biospectroscopy, Vibrational Spectroscopy
Biomedical
Biospectroscopy
Clinical Biophotonics
Computational Imaging, Sensing and Diagnostics

My research focuses on the use of computation 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 new 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. The FOV of these computational microscopes is equal to the active-area of the sensor-array, easily reaching >20mm² or >10cm² by employing 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 applications in global health. Through the development of similar computational imagers, I will also report the discovery of new 3D swimming patterns observed in human and animal sperm. Shedding light onto the statistics and biophysics of various micro-swimmers’ 3D motion, these results provide an important example of how biomedical imaging significantly benefits from emerging computational algorithms/theories, revolutionizing existing tools for observing various micro- and nano-scale phenomena in innovative, high-throughput, and yet cost-effective ways.

Keywords: Imaging, Lab-on-a-Chip/Microfluidics, Portable Instruments, Sensors
Application Code: Biomedical
Methodology Code: Microscopy
The diagnosis of Alzheimer’s disease is cumbersome and often delayed by lack of a simple diagnostic tool such as a blood test. In this study, we applied near infrared (NIR) Raman microspectroscopy and surface enhanced Raman spectroscopy (SERS) coupled with advanced multivariate statistics for the differential diagnosis of AD based on blood serum. We analyzed NIR Raman spectral data from patients diagnosed with AD, patients diagnosed with other types of dementia (OD) and Healthy Control (HC) subjects. Artificial neural networks (ANN) and a support vector machine (SVM) were utilized for spectral data analysis. A Raman spectrum of blood serum represents the total biochemical composition of the fluid, subtle and specific changes of which could reflect a specific disease. We found that advanced statistical analysis of the serum Raman spectra allows for differentiating AD, OD and HC subjects with more than 95% sensitivity and specificity. Further study of a much larger cohort is required for the validation of the method and for establishing its effectiveness for early disease diagnostics. When fully developed, this fast, inexpensive noninvasive method could be used for screening of at risk patient populations for AD development and progression.

**Keywords:** Biomedical, Chemometrics, Raman Spectroscopy, Statistical Data Analysis

**Application Code:** Biomedical

**Methodology Code:** Biospectroscopy
The surgeon’s limited ability to accurately delineate the tumor margin during surgical interventions is one key challenge in clinical management of cancer. New methods for guiding tumor resection decisions are needed. Numerous studies have shown that tissue autofluorescence properties have the potential to assess biochemical features associated with distinct pathologies in tissue and to distinguish various cancers from normal tissues. However, despite these promising reports, autofluorescence techniques were sparsely adopted in clinical settings. Moreover, when adopted they were primarily used for pre-operative diagnosis rather than guiding interventions. To address this need, we have researched and engineered instrumentation that utilizes label-free fluorescence lifetime contrast to characterize tissue biochemical features in vivo in patients and methodologies conducive to real-time (few seconds) diagnosis of tissue pathologies during surgical procedures. This presentation overviews clinically-compatible multispectral fluorescence lifetime imaging techniques developed in our laboratory and their ability to operate as stand-alone tools, integrated in a biopsy needle and in conjunction with the da Vinci surgical robot. We present pre-clinical and clinical studies in patients that demonstrate the potential of these techniques for intraoperative assessment of brain tumors and head and neck cancer. Current results demonstrate that intrinsic fluorescence signals can provide useful contrast for delineation distinct types of tissues including tumors intraoperatively. Challenges and solutions in the clinical implementation of these techniques are discussed.
The Application of Matrix Effect Factor (MEF) for High Throughput Cosmetics Analysis by LC-MS

Although LC-MS methods are sensitive and selective analytical techniques, they often suffer from matrix effects, which can be observed as either a loss (ion suppression) or an increase (ion enhancement) in responses. The matrix effects affect the detection capability, precision and/or accuracy for the analytes of interest. Thus, the matrix effects should be evaluated during method development by comparing the response of a standard solution prepared in a sample matrix over the response in neat solutions or by comparing the calibration-curve slope of standard solutions in sample matrix over the slope of standards in neat solutions. Since no representative matrix is available for cosmetic samples with unique ingredients for each sample, how to evaluate and minimize the matrix effects is a great challenge. Different techniques to minimize matrix effects will be presented. The concept of matrix effect factor (MEF) will be introduced, and the development of a Microsoft Excel template to calculate the MEF for high throughput cosmetics analysis by LC-MS will be discussed.

Keywords: Cosmetic, High Throughput Chemical Analysis, Liquid Chromatography/Mass Spectroscopy, Sample P
Application Code: Consumer Products
Methodology Code: Liquid Chromatography/Mass Spectrometry
### Abstract Text

Flame-induced atmospheric pressure chemical ionization (FAPCI) is a novel ionization method, generating metal ions and other charged species by a flame to ionize analytes through ion-molecule reactions (IMRs). It has been demonstrated for the analysis of organic compounds, where protonated and metal-adducted analyte ions were detected. In this study, thermal desorption (TD) was combined with FAPCI/MS to characterize volatile and semi-volatile compounds. The toxic compounds such as preservatives in cosmetics and pesticides on fruits were direct detected by TD-FAPCI/MS.

The TD-FAPCI consisted of a heated oven and an oxyacetylene flame. A stainless steel probe was used to scrape the fruit or contact the cream surface for sampling. After that, the probe was inserted into the heated oven for thermal evaporation. A stream of heated nitrogen gas carried analyte vapors from the heated oven to the ionization region for IMRs with charged species generated in the flame. The metal solutions were also introduced into the flame to ionize analytes with different metal ions. A triplet quadruple and a quadruple time-of-flight mass analyzer were used to characterize the analyte ions.

The use of TD-FAPCI/MS for direct detection of preservatives such as DMDM hydantoin, methylisothiazolinone, phenoxyethanol in various cosmetic products (including lotions, creams, foams, gels, and sprays) were also demonstrated. The detection limit of preservatives in cosmetic products were far below the legal limit of their respective concentrations. The analysis of pesticides on fruits and vegetables was also demonstrated without visible sample damage. Without extraction and separation processes, the analysis of each sample is completed in 15 s. In conclusion, TD-FAPCI/MS is a sensitive, reproducible, quantitative, and rapid technique; it can be used for large-scale screening and high throughput analysis of cosmetics, vegetables, and fruits to assure consumer’s health.

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

**Application Code:** Food Science

**Methodology Code:** Mass Spectrometry
Food safety is increasingly a very visible public concern in view of sourcing of food ingredients from around the world. The demand for food in 2030 is expected to increase by 50% of current rate of consumption. The increasing demand for food is expected to be met by an increase in food trade and the accompanying globalization of supply chains. The globalization of food trade brings food safety concerns such as pathogen outbreaks and the presence of chemical residues in food. In addition, economically motivated adulteration of food ingredients, as in melamine adulteration of milk in 2008, can pose serious harm to consumers. The projected increase in food trade and the accompanying food safety risks pose a significant burden on both regulators and the public alike.

Analytical testing is the frontline defense in ensuring the safety of food ingredients traded and sold around the world. Sensitive analytical techniques such as LC-MS are a powerful means to detect the presence of chemical residues in food and enable the safety of food commodities. The challenge lies in being able to rapidly analyze a large number of food ingredients at the point of origin and help detect potential safety issues at the source.

Although there is no single analytical solution, recent developments of compact, simpler mass spectrometers coupled with novel ambient ionization inlet systems presents a potential opportunity to provide a preliminary screening test for expected or targeted chemicals at a point-of-origin location. This presentation will show simple front-end sample treatments ranging from no sample preparation to relatively simple sample preparation techniques (such as SPME) coupled with sample inlet systems such as ASAP, SPME/ASAP, Open Port Source Inlet (OPSI) and of course LC/MS using a single quadrupole atmospheric pressure ionization (API) mass spectrometer. A variety of representative applications will be described.

Keywords: Food Science, Forensic Chemistry, Mass Spectrometry
Application Code: Food Contaminants
Methodology Code: Mass Spectrometry
### Abstract Text

Ensuring food and water safety requires the identification and determination of important contaminants of concern. This presentation focuses on arsenic species and N-chloro-organics in water and food. Human exposure to high concentrations of arsenic from water and food is associated with increased risk of developing several cancers and causing various adverse health effects. A variety of arsenic species can be present in food, and these arsenic species have very different toxicities. It is necessary to identify and quantify individual arsenic species. We describe several methods for the determination of arsenic species in food items of common consumption, such as fish, chicken meat, rice, and vegetables. These methods involve enzyme-assisted digestion of samples, high performance liquid chromatography (HPLC) separation of arsenic species, and simultaneous detection with both inductively coupled plasma mass spectrometry (ICPMS) and electrospray ionization tandem mass spectrometry (ESI-MS/MS). These methods allow for the identification and determination of more than 20 arsenic species, most often including inorganic arsenicals, monomethylarsenicals, dimethylarsenicals, arsenobetaine, 3-amino-4-hydroxyphenylarsonic acid (Roxarsone) and its metabolites. The arsenic speciation information enables meaningful assessment of human exposure to arsenic species from food consumption.

The second component of this presentation focuses on the identification of emerging disinfection byproducts (DBPs). N-chloro-organics likely represent a substantial proportion of the currently unidentified mass of halogenated DBPs. Using HPLC and high resolution tandem mass spectrometry, we demonstrate the identification of several new N-chloro-organics in treated water. We further studied the formation of these DBPs and identified their precursors. The improved understanding provides sound basis for minimizing the formation of these emerging DBPs, contributing to water safety.

### Keywords
- Environmental/Biological Samples, Food Safety, HPLC, ICP-MS
- Food Safety
- Liquid Chromatography/Mass Spectrometry
## Session Title
Identification and High Throughput Analysis for Food Safety and Cosmetics

## Abstract Title
Fast, Automatic, and Accurate Determination and Identification of Targeted Analytes in High-Throughput Analysis by Chromatography – Tandem Mass Spectrometry

## Primary Author
Steven Lehotay
USDA ARS ERRC

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### Abstract Text
Although non-targeted identification of unknown contaminants in foods and the environment is desirable in some applications, which gains much attention in vendor marketing and the scientific media, the great bulk of real-world analyses involve targeted pesticides, environmental contaminants, veterinary drugs, and other known chemicals of concern. Sample throughput and laboratory efficiency is most significantly increased by combining as many analytes and applications into a single analysis in what has become known as a mega-method. The newest tandem mass spectrometry (MS/MS) instruments [quadrupole/high resolution MS (Q/HRMS) or triple quadrupole (QQQ)] can acquire data very fast with high sensitivity, which allows targeted ultra-trace analysis of several hundreds of analytes separated by gas and liquid chromatography (GC and LC). High-throughput is achieved by batch extraction using a “quick, easy, cheap, effective, rugged, and safe” (QuEChERS) approach followed by fully automated cleanup and analysis in parallel using mini-cartridge solid-phase extraction (SPE) with a robotic liquid handling autosampler. Low-pressure (LP) GC-QQQ separates >200 pesticides and environmental contaminants in <10 min, and ultra-high performance (UHP) LC-QQQ matches speed and data quality when only using filtration of QuEChERS extracts for cleanup. Automatic summation function chromatographic peak integration and post-run data processing yields accurate quantification and identification of analytes with very little need for human review of results. We have validated this high-throughput concept in different food analysis applications.

### Keywords:
Automation, Chromatography, Food Contaminants, High Throughput Chemical Analysis

### Application Code:
Food Contaminants

### Methodology Code:
Chemical Methods
KWJ and partner SPEC-Sensors have fabricated a family of miniature, yet high-performance, gas sensors for air quality (indoor and outdoor) and wearable personal exposure monitoring. These sensors bridge the cost-performance-gap. Distributed high volume environmental and wearable gas sensing is now happening enabled by these new devices. KWJ’s innovative approach combines printed electronics manufacturing capability with new nano-electro-catalysts for gas sensing to enable a disruptive sensor platforms that allow monitoring applications for many important gases including: CO, alcohol, O2, O3, NO, NO2, SO2, H2S, Cl2, and H2 at health, safety, and environmentally significant levels in the field.

The WHO has reported more than 5 million deaths per year from air pollution worldwide and air quality is the 4th largest contributor to the earth’s human disease burden. One cannot control or avoid something that cannot be seen or measured and sensors for indoor and outdoor air quality are our window to understanding and mitigation of air quality and a revolution could arrest or even reverse climate change for the benefit of everyone everywhere. Economic driver for deployment of billions of low cost sensors are developing as is the technology for widespread low cost tiny air quality monitors to enable smart cities and wearable technology for air quality parameters. These co-emerging factors of increased need, available sensor platforms AND availability of high-volume low cost miniature gas sensors combine to enable the deployment of billions of sensors for environmental awareness and human health and safety as well disaster relief situations. Laboratory and field testing data are presented here for the tiny SPEC sensors and KWJ platforms that validate performance for sub-ppm level detection and monitoring applications for CO and NO2 and Ozone in air quality. Novel electrolytes [RTILs] and O3 sensors with 200 ppt [0.2 ppb] limit of detection are demonstrated.

Keywords: Electrochemistry, Environmental/Air, Sensors
Application Code: Environmental
Methodology Code: Sensors
Worldwide, exposure to airborne pollutants and explosive gases represents an increasing danger to human health and safety. New tools to monitor gaseous hazards in real time and at the point of personal exposure are needed for preventative awareness. To address this need, our team is developing a wearable gas sensing platform featuring microfabricated electrochemical sensor arrays and embedded instrumentation to enable a miniaturized and low cost solution for personal exposure monitoring. The robust electrochemical gas sensors feature room temperature ionic liquid (RTIL) electrolytes and carefully selected electrochemical methods for rapid measurement of several target gases within a real-world mixed-gas environment. To permit wearable implementation of the entire sensory system, a multi-method electrochemical instrumentation module has been realized using custom analog microelectronics and a microcontroller for signal processing and communication. Good sensitivity, linearity and repeatability have been demonstrated for oxygen, methane, and several air pollutants. The highly integrated system is well suited for rapid measurement of hazardous gases and improved awareness of personal exposures that facilitate preventative action.

Abstract Text

Keywords: Electrochemistry, Gas, Instrumentation, Sensors
Application Code: Safety
Methodology Code: Sensors
Ionic liquids (ILs) are attractive solvents for electrochemical processes due to their favourable physical properties but their uptake is limited, partially because their Stern layer nanostructure is poorly understood compared to molecular solvents. Here in situ amplitude modulated - atomic force microscopy (AM-AFM) has been used to reveal the Stern layer nanostructure of the 1-ethyl-3-methylimidazolium bis(trifluoromethyl-sulfonyl)imide (EMIm TFSI) - HOPG interface with molecular resolution. The effect of applied surface potential and added 0.1 wt/wt% LiTFSI or EMImCl on ion arrangements is probed between ±1 V. For pure EMIm TFSI at open circuit potential, well defined rows are present on the surface formed by an anion-cation-cation-anion (A-C-C-A) unit cell adsorbed with like ions adjacent. As the surface potential is changed the relative concentrations of cations and anions in the Stern layer responds, and markedly different lateral ion arrangements ensue. The changes in Stern layer structure at positive and negative potentials are not symmetrical due the different surface affinities and packing constraints of cations and anions. When Li+ or Cl- is present some Stern layer ionic liquid cations or anions (respectively) are displaced producing starkly different structures.

**Keywords:** Atomic Force Microscopy (AFM), Electrochemistry

**Application Code:** Material Science

**Methodology Code:** Microscopy
Conventional synthesis of functional materials relies heavily on water and organic solvents. Alternatively, the synthesis of functional materials using, or in the presence of, ionic liquids represents a burgeoning direction in materials chemistry.[1] Ionic liquids are a family of non-conventional molten salts that can act as both templates and precursors to functional materials, as well as solvent. They offer many advantages, such as negligible vapor pressures, wide liquidus ranges, good thermal stability, tunable solubility of both organic and inorganic molecules, and much synthetic flexibility. The unique solvation environment of these ionic liquids provides new reaction media for controlling formation of porous materials and tailoring morphologies of advanced materials. The resulting materials can be used in gas separation, sensing, and energy storage. Challenges and opportunities in this research area will be discussed.

Acknowledgments: This work was supported by the Division of Chemical Sciences, Geosciences and Biosciences, Office of Basic Energy Sciences, U.S. Department of Energy, under contract No. DE-AC05-00OR22725 with UT-Battelle, LLC.


Keywords: Sensors, Separation Sciences
Application Code: Material Science
Methodology Code: Electrochemistry
Ionic liquids (ILs) composed of organic cations or anions are widely employed in various chemical and electrochemical applications such as solvents, catalysts, sensors, fuel cells and batteries. They are typically more electrochemically stable than those aqueous electrolytes. Moreover, in the presence of electric fields, the structures and properties of an IL at the electrode interface resemble crystalline solids that are significantly different from traditional electric double layers. This presentation will discuss the fundamental and applied IL electrochemistry research and their applications in electroanalysis and electrocatalysis in our laboratory. Several examples will be discussed to demonstrate new approaches of utilizing the unique properties of ILs not only as solvents and electrolytes for electrochemical gas sensor development but also as an active medium to in situ generate electrocatalysts. In the first part of the talk, we will illustrate the new redox mechanisms of common gases (i.e. CH4, SO2, H2, O2) and small organic molecules (methanol and formaldehyde) in the ILs and how they are used to develop new electroanalytical methods that enable gas detections in ambient conditions. In the second part, we will discuss examples that the electrochemical activity of the constituent ions of an IL can be utilized to generate new catalysts at electrode interface with high catalytic activity as well as good selectivity for hydrocarbon (methanol and methane) as well as hydrogen oxidation.

Keywords: Electrochemistry, Electrode Surfaces, Energy, Environmental/Air
Application Code: Environmental
Methodology Code: Electrochemistry
Chemical and biological sensors – especially those that operate label-free – represent an extremely popular area of research for chemists, engineers, biologists, and physicists. But while sensing is an important part of the problem, it is not the only part of the problem – in many real-world applications, samples must be extensively processed prior to analysis (with sensors or other detection schemes). Microfluidics has been used widely to integrate sample processing with sensors – such devices typically rely on a format in which fluids are pumped through enclosed channels on a planar substrate. I will describe our work using an alternative format known as “digital microfluidics,” in which droplets are manipulated by applying electrostatic forces to an array of electrodes coated with a hydrophobic insulator. I will review three types of integrated sensors that we have developed, including impedance sensors for live adherent cell counting, voltammetry and related techniques for bulk solution measurements, nuclear magnetic resonance for reaction monitoring, and mass spectrometry for quantification of illicit and prescription drugs in complex matrices. I will make the case that these examples (and others) confirm that digital microfluidics is a useful new tool for integrating bioanalysis with sample processing, with potential for application to a large number of real-world problems.
Plasmonic nanomaterial promises two highly attractive features in one entity: large surface area and local field enhancement. However, most colloidal nanoparticles and lithographically patterned nanostructures lack architectural hierarchy. Recently, our group has fabricated a novel class of nanoporous gold (NPG) nanoparticle, featuring large per-particle surface area and high-density plasmonic hot spots. By forming NPG nanoparticles directly on a substrate, NPG array (NPGA) features high uniformity and reproducibility. In addition to fundamental understanding of NPGA, we have made significant progress in developing surface-enhanced spectroscopy for analytical sensing such as surface-enhanced Raman scattering (SERS), localized surface plasmon resonance (LSPR) spectroscopy, surface-enhanced fluorescence (SEF), and surface-enhanced near-infrared absorption spectroscopy (SENIRA).
A multitude of important biomolecular processes occur at the cell membrane, including those that involve membrane proteins, lipids, and soluble factors. We have developed a robust array-based approach to simultaneously characterizing multi-factorial biomolecular interactions by integrating Nanodisc technology with multiplexable silicon photonic sensor arrays. Focusing on interactions relevant to blood coagulation, we demonstrate the applicability of this platform to rapidly determine relative binding interactions between a number of coagulation factors with Nanodiscs with varying lipid composition in both the presence and absence of the membrane protein Tissue Factor. By parsing out both lipid and membrane contributions to factor binding and activity we hope to parse out subtle details that provide elegant control over normal blood coagulation as well as dysfunction that leads to both bleeding and clotting disorders.

**Abstract Text**

A multitude of important biomolecular processes occur at the cell membrane, including those that involve membrane proteins, lipids, and soluble factors. We have developed a robust array-based approach to simultaneously characterizing multi-factorial biomolecular interactions by integrating Nanodisc technology with multiplexable silicon photonic sensor arrays. Focusing on interactions relevant to blood coagulation, we demonstrate the applicability of this platform to rapidly determine relative binding interactions between a number of coagulation factors with Nanodiscs with varying lipid composition in both the presence and absence of the membrane protein Tissue Factor. By parsing out both lipid and membrane contributions to factor binding and activity we hope to parse out subtle details that provide elegant control over normal blood coagulation as well as dysfunction that leads to both bleeding and clotting disorders.

**Keywords:** Bioanalytical, Lipids, Membrane, Protein

**Application Code:** Bioanalytical

**Methodology Code:** Sensors
Microfluidic Sample Preparation, Separation and Delivery for Ultrasensitive MS-Based Bioanalyses

Mass spectrometry (MS) provides information-rich, label-free analysis of biomolecules and is a workhorse technology in the fields of proteomics and metabolomics. Coupling microfluidics with MS enables new applications and a reduction in required sample sizes. For example, active microfluidic devices with integrated pneumatic microvalves allow automated, multistep biochemical analyses to be performed using subnanoliter volumes of sample and reagents. Additionally, robotically addressed, photolithographically patterned nanowells can be used to directly miniaturize existing “one-pot” proteomic sample preparation workflows, dramatically reducing losses of precious samples to surfaces. We will describe microfluidic interfaces for electrospray ionization (ESI)-MS that enable stable operation at low-nanoliter-per-minute flow rates, enabling sub-attomole detection limits. We will then describe the coupling of droplet-based, droplet-free, and nanowell platforms with optimized ESI interfaces and their application to (1) real-time monitoring of high-rate, solution-phase reactions, (2) preconcentration, injection and capillary electrophoresis separation of protein and peptide mixtures, and (3) preparation, separation and analysis of ultrasmall proteomic samples.

Keywords: Electrospray, Lab-on-a-Chip/Microfluidics, Mass Spectrometry, Proteomics
Application Code: Genomics, Proteomics and Other ‘Omics
Methodology Code: Microfluidics/Lab-on-a-Chip
Microfluidics provides nowadays a huge toolbox for bioanalytical and biological research. A great potential of microfluidics for analytical applications lays in the possibility to create small liquid environments of defined and reproducible volumes so that analyses can be performed with little sample and in unprecedented high sensitivity. We use two approaches to confine liquids in volumes ranging from pL to nL; (i) valve systems in multilayer microdevices made of silicone elastomer (PDMS) and (ii) droplet microfluidics in continuous and static modes.

The first valve-based systems proved highly useful for questions in the field of single cell analysis, solving challenges such as single-cell trapping, cell cultivation and implementation of washing and incubation steps. Furthermore, in combination with powerful detection methods such as the ELISA (enzyme-linked immunosorbent assay), we could quantify proteins and metabolites present in individual cells in amol levels. Recent developments and modifications of this versatile method will be shown. The alternative microdroplet approach is optimal for large throughput analysis of liquid samples, with fluorescence readout or label-free detection by mass spectrometry. We recently developed new methods to interface droplet microfluidics with MALDI MS and ICP-MS. The use of these interfaces is demonstrated for the analysis of single cells, and also for the identification of post-translational modification of proteins.

Keywords: Bioanalytical, Lab-on-a-Chip/Microfluidics, Mass Spectrometry, Proteomics
Application Code: Bioanalytical
Methodology Code: Microfluidics/Lab-on-a-Chip
This presentation describes a set of new scan methods which are especially useful for miniature mass spectrometers. The methods emphasize ac scans over rf scans and they allow various types of MS/MS experiments to be implemented in a single ion trap mass analyzer. These experiments include precursor, neutral loss and multiple reaction monitoring scans. In addition, simple single stage mass spectra are performed with increased resolution and sensitivity using successive ac resonance signals applied at arbitrary frequencies. Linear mass scans and extended mass ranges are amongst the characteristics which are illustrated. Data are shown for a variety of samples of forensic and biological interest.

Keywords: Analysis, Ion Trap, Mass Spectrometry, Portable Instruments
Application Code: Bioanalytical
Methodology Code: Mass Spectrometry
Miniature Mass Spectrometers

High Pressure Mass Spectrometry: A Path to Handheld Analyzers with Specificity and Sensitivity

Mass spectrometry (MS) is an information rich chemical measurement technique that also displays high sensitivity. Arguably, mass spectrometry is the most informative of all of the techniques that the chemical analyst has in their toolbox. There has been interest in portable mass spectrometry for decades due to the ability to uniquely identify a compound at low concentrations, but the need to perform MS under low-pressure conditions has limited the size, weight, and power of portable mass spectrometers with masses exceeding 10 kg. We have been exploring the miniaturization of Paul trap mass analyzers for nearly two decades due to the attractive scaling of performance metrics with reduced dimensions. Specifically, resolving power is fundamentally independent of length scale and charge capacity scales with linear dimension rather than trap volume. Moreover we discovered that mass spectrometry could be performed at unprecedented pressures using small ion traps. Our work has focused on ion traps with fundamental dimensions in the 100 to 500-µm range. We define High Pressure MS (HPMS) to be mass spectrometry performed at pressures exceeding 0.1 Torr. Recent advances including ion source options and MS/MS capabilities will be discussed.

Keywords: Forensics, Mass Spectrometry, Process Control, Tandem Mass Spec
Application Code: Homeland Security/Forensics
Methodology Code: Mass Spectrometry
We report on a novel miniaturized linear ion trap made using a series of wires held between two support plates. The wire ion trap is made by replacing each of the four hyperbolic electrodes of a conventional linear ion trap with 4-6 wires of 80-120 microns’ diameter. Wires are accurately positioned and held taut between two printed circuit boards or other rigid substrates. Holes in the substrates can be laser drilled for accurate placement. This approach takes advantage of the high accuracy of 2-dimensional positioning but allows a trap of arbitrary length, improving trapping capacity. The performance of the trap is largely immune to problems associated with mechanical misalignment, addressing a major issue affecting miniaturized ion traps. For instance, even if the support plates are 2-3 degrees off of parallel, the mass resolution is not noticeably degraded. In addition, the trap is extremely lightweight and rugged. The trap can be dropped from several feet and not require re-alignment. Experiments with pure compounds and complex mixtures show typical peak widths of 0.3 amu and detection limits in the ppb range.

**Keywords:** Instrumentation, Mass Spectrometry, Quadrupole MS, Tandem Mass Spec

**Application Code:** Other

**Methodology Code:** Mass Spectrometry
This session will include a brief survey followed by demonstrations of the integrated miniature mass spectrometry (MS) systems by manufacturers or academic researchers. The main commercial or research team will be invited to bring their product or prototype systems to the session to provide detailed introductions to the audience. The systems include the portable and transportable MS systems that are suitable for analysis of gaseous, liquid and solid samples. The types of instruments include ion trap, mass filter and time-of-flight. Direct sampling with internal ionization, gas chromatography with internal ionization and sample cartridges designed based on ambient ionization are used for sample introduction. This session will provide an unique opportunity for the audience to see the devices and to have direct interactions with the designers of the miniature MS systems.

Keywords: Analysis, Bioanalytical, Mass Spectrometry, Sampling
Application Code: Biomedical
Methodology Code: Mass Spectrometry
Drugs, Environmental/Biological Samples, Forensics, Mass Spectrometry

Application Code: Homeland Security/Forensics

Methodology Code: Mass Spectrometry

Abstract Text

With an increasing number of states legalizing marijuana for recreational purposes, new field-based detection modalities are necessary to conclusively establish acute exposure. Whether the motivation be for law enforcement, point-of-care diagnostics, or work-place safety chemical detection and engineering solutions are necessary to realize rapid detection of marijuana in the field. To address this challenge in Washington State, we have initiated a set of experiments using institutional review board approved volunteers to collect breath, oral fluids, and, in select cases, blood to establish the scope of the challenge. In addition to evaluating a suite of different breath collection methods, we have explored different mechanisms to release THC into the vapor phase and enable its detection using technologies that exploit gas-phase ion mobility. In addition to characterizing the performance of a fielded differential mobility spectrometer system, we have sought to validate its performance by conducting parallel sets of experiments for direct THC quantitation using UPLC/mass spectrometry. While the development of a fielded system is the ultimate goal, these experiments also seek to capture a range of consumption patterns, including casual and chronic smokers, using modern, commercially available, strains of marijuana. Using this tandem approach, we have established the, albeit decreasing, levels of THC found in breath and oral fluids up to two hours post smoking. These data include a range of volunteers to capture intra and inter-subject variability. Questions related to the exact levels of THC consumption that conclusively indicate impairment remain, however, our initial results demonstrate that sufficient THC is available for non-invasive sampling within the few hours immediately after smoking to enable detection. Quantitative values for THC present across the range of samples collected along with initial results from field testing will be presented.

Keywords: Drugs, Environmental/Biological Samples, Forensics, Mass Spectrometry
### Session Title
Drug Detection in the Field

### Abstract Title
Detection of Drugs of Abuse and Forensic Attribution Using Raman Spectroscopy and Existing Military Chemical Detection Equipment

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<td>Jason Guicheteau</td>
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<td>USA RDECOM Edgewood Chemical Biological Center</td>
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### Abstract Text
The U.S. Army Edgewood Chemical Biological Center is developing and demonstrating detection of illicit drugs of abuse, e.g., marijuana, cocaine, heroin, and designer drugs, using existing chemical detection systems and integrating illicit drug detection into the family of Chemical Biological Radiological Nuclear and Explosives (CBRNE) sensors. This work introduces the Chemical Fingerprint Identification System (CFIS) as well as modifications to current military detection equipment to reduce the overall number of types of detection equipment fielded; saving purchase and maintenance cost, as well as training time.

### Keywords:  
Chemical Ionization MS, Detection, Raman Spectroscopy, Surface Enhanced Raman Spectroscopy

### Application Code:  
Homeland Security/Forensics

### Methodology Code:  
Sensors
The reliable testing of drug consumption e.g. for traffic control is still challenging as different drugs (alcohol, Marihuana, heroin, cocaine, amphetamines and others) play significant role. While hand-held, valid and rapid alcohol test equipment is available since many years, other drugs still are controlled using test stripes for sweat, saliva or urine which is time consuming and requires thorough control of sampling. Furthermore, significant numbers of false positives can be observed.

Therefore, we investigated the potential of comprehensive analysis of human breath for the simultaneous detection of the most common drugs using ion mobility spectrometry coupled to rapid gas-chromatographic pre-separation (GC-IMS). The detection of Marihuana consumption using GC-IMS was already demonstrated but now studies on the pharmacokinetics as well as correlations to the blood THC level – still the measure for legal thresholds – are available. Furthermore, the simultaneous detection of other drugs such as alcohol was investigated.

Abstract Text

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Drug Detection in the Field

Detection of Drugs with Cantilever-Enhanced Photoacoustic Spectroscopy

Cantilever-enhanced photoacoustic spectroscopy (CEPAS) in mid-infrared region was applied in detecting drugs with different detection schemes both in gas- and solid phase. A patented cantilever sensor operating in non-resonant mode was used as a pressure sensor in photoacoustic detection to achieve an ultimate sensitivity and flexibility in different applications (1,2).

In gas-phase, trace gas detection of volatile organic compounds (VOC) originating from drugs and drug precursors was studied. Widely scanning external cavity quantum cascade lasers (EC-QCL) were used in conjunction with PAS for multi-component gas detection. QCLs can be constructed in relatively high output powers in the mid-infrared region, which converts straight into lower detection limits in the photoacoustic detection. Ppb-level detection limits for different VOCs were achieved with PA201 photoacoustic detector (Gasera, Ltd.) by using different QCL sources.

In solid-phase, detection of cocaine use by using hair samples was investigated. A Fourier transform infrared (FTIR) spectrometer was used in conjunction with PA301 photoacoustic detector for solids and liquids (Gasera, Ltd.). Hair samples of cocaine users were successfully separated by their spectra from a reference group with no history of cocaine. Further, detection of tetrahydrocannabinol (THC) levels in cannabis samples was studied with the same measurement setup. Also, studies using an EC-QCL as an infrared source for PA301 were performed to achieve even higher signal-to-noise level in the photoacoustic detection.

Fig 1. Vaporized methyl benzoate sample measured with EC-QCL PAS setup.


Keywords: Drugs, Infrared and Raman, Photoacoustic, Vibrational Spectroscopy

Application Code: Homeland Security/Forensics

Methodology Code: Vibrational Spectroscopy
Drug Detection in the Field

High Performance Ion Mobility Spectrometry for Accurate Chemical Identification in the Field

Compared to conventional HPLC and GC based analytical methods, ion mobility spectrometry (IMS) offer significant higher speed and intrinsic simplicity in field analytical applications. However, owing to the poor resolution common IMS products, it is only used as the first step screening device, subsequently, a confirmation step are required to form a chemical identification. In this study, we have significant improved the IMS resolution to resolve interfering co-existing chemical in the field operation in order to achieve accurate chemical identification. With the newly developed high performance IMS (HPIMS), it resolving power is comparable to common HPLC systems. HPIMS has been used for field analytical testing of controlled substances, pharmaceutical drugs, especially used to resolve isomeric chemicals. HPIMS with an electrospray ionization (ESI) source has proven adept at detecting a wide range of illegal drug compounds. The well-known psychoactive ingredient 9-tetrahydrocannabinol (THC) found in the plant is fat soluble after consumption, ultimately metabolized and oxidized to the inactive 11-nor-9-THC acidic form that is pharmacologically inactive. Our initial studies have been extended to focus on identification of THC and related compounds in matrix using ESI-HPIMS with direct ionization from a sample syringe aimed at an alternative rapid quantitative screening for cannabis use.

Keywords: Detection, Forensic Chemistry, HPLC, Identification

Application Code: High-Throughput Chemical Analysis

Methodology Code: Liquid Chromatography
### Abstract Text

Abuse of drugs including new psychoactive substances is a global problem. To enforce strict regulations on those drugs, reliable screening in field is expected to be one of the effective solutions. Since immunochemical methods, which are commonly used in such screening, cannot identify several hundreds of drugs, alternative methods are required. We therefore developed a portable mass spectrometer (MS), which was characterized by a high sensitive ionization source, a low pressure dielectric barrier discharge ionization source and a compact linear ion trap mass analyzer. For liquid sample analyses, we developed a vacuumed headspace technique in which sample molecules are concentrated in the headspace in order to improve the sensitivity of our instrument. For solid sample analyses, we developed a probe heating method that conducts rapid sampling and vaporizing of solid samples with low power consumption. We confirmed that the sensitivity of our portable MS was high enough to detect 0.1 ppm methamphetamine in urine as well as 1 ng methamphetamine powder. In addition, by creating the mass and tandem mass spectra data base, several tens of drugs were detected. Those results suggest that the portable MS has a capability to perform the drug screening in the field.

This work was partially supported by “R&D Program for Implementation of Anti-Crime and Anti-Terrorism Technologies for a Safe and Secure Society”, Strategic Funds for the Promotion of Science and Technology of the Ministry of Education, Culture, Sports, Science and Technology, the Japanese Government.

### Keywords
- Drugs
- Forensic Chemistry
- Mass Spectrometry

### Application Code
- Homeland Security/Forensics

### Methodology Code
- Mass Spectrometry
Designer and emergent illicit drugs have entered the market at a rapid pace in the past years, and synthetic cathinones (aka: "bath salts") are one example of these drugs. Synthetic cathinones are the beta-keto phenethylamine derivatives, that produce pharmacological effects similar to the Schedule I substances such as cathinone, methcathinone, and 3,4-methylenedioxymethamphetamine (MDMA). The parent cathinone structure is easily derivatized at any of four sites to generate analogues not yet regulated by state legislation or the DEA Scheduled substance list. Unfortunately, the derivatization of the cathinone structure has resulted in presumptive color tests that are often not detecting new bath salts or are providing different colors for different cathinone derivatives or require several vials of various component reagents to be effective. Furthermore, color tests inherit the possibility of false positive. In this study, an electrochemistry technique, cyclic voltammetry, was used to analyze synthetic cathinones. The results include the electrocatalysts, pH effect, and the interference effects used for screening the synthetic cathinones.
Drug Detection in the Field

Paper-Based Diagnostic Devices in the Hands of Untrained Users

Paper-based fluidic devices, also known as microPADS, have been described as a promising platform for the development of point-of-care diagnostic assays for use in the field. MicroPADs are inexpensive to fabricate, they are typically small and portable, and they are claimed to be simple to operate, but how accurate and precise are paper-based diagnostic tests in the hands of untrained users? This presentation will describe our work on the development of a simple paper-based test for glucose as well as our experiences with introducing microPADs into a general chemistry laboratory course for first-year college students. Over 100 students prepared and then performed paper-based tests for glucose and quantified the results using a smart phone and a 3-point external calibration curve. We then evaluated the accuracy and precision of the results to determine whether paper-based diagnostic devices can indeed deliver on their promise to be useful in the hands of untrained users.

Keywords: Biomedical, Biosensors, Lab-on-a-Chip/Microfluidics

Application Code: Biomedical

Methodology Code: Microfluidics/Lab-on-a-Chip
Extractable and Leachable Studies of Parenteral Infusion and Transfusion Products

This presentation describes the general extractable/leachable study practices of parenteral infusion and transfusion products. It includes (1) extractable and leachable study designs and extractable/leachable study work flow using a wide variety of analytical techniques (UPLC/UV/MS, GC/MS, ICP and TOC). (2) the determinations of concentration thresholds for extractable and leachable that require identification and quantification (3) risk assessments of the extractable and leachable compounds and (4). Selection and analytical method development/validation for target leachable compounds that are monitored for the stability evaluation of infusion solutions.

Keywords: Gas Chromatography/Mass Spectrometry, ICP-MS, Liquid Chromatography/Mass Spectroscopy, Mat
Application Code: Other
Methodology Code: Mass Spectrometry
As drug formulations move from small molecule to Biologics, the impact extractable and leachable compounds have on product safety continues to increase, from manufacturing (single-use-systems) to final product. Being able to detect potential E/L compounds continues to be a challenge due to the lack of standardized methods and protocols.

Extractable and leachable analysis routinely use GC/MS and LC/MS with various ionization methods. For GCMS analysis, many standard methods exist with identification using commercially available EI libraries (NIST, Wiley). For LCMS analysis, identification limited by the lack of commercially available LC/MS and MS/MS database. To enhance identification of potential E/L compounds, a new accurate mass E & L commercial database has been developed that contains over 1000 compounds, with over 300 compounds with MS/MS spectra, obtained with multiple collision energies, multiple adducts and positive and negative ion polarity. The database also contains information on classification (compound type, ELSIE presence), safety information, CAS Number, ChemSpider ID, Chinese name, SWISS Ordinance and reference information.

Extraction profile of components from a meter dose inhaler were prepared and analyzed using a quadrupole-time-of-flight mass spectrometer using and both Electrospray and APCI, both positive and negative ionization, and with both AllIons and Data Dependent MS/MS data acquisition methods.

From AllIons MS/MS acquisition, the data can be mined directly using Find by Formula referring to the E&L PCDL (database and MS/MS library) as that formula source, and qualifying proposed compounds via identification of fragments referenced to the PCDL MS/MS library entries. The E&L PCDL was also used for database and library searching of data generated from Target MS/MS of compounds found to differentiate samples via statistical analysis; Mass Profiler for 2 sample comparison and Mass Profiler Professional for multi-variate analysis.
During routine screening studies for extractables and leachables analysis, there are often compounds that are unidentified or only partially characterized. Unidentified extractables can result from new or uncommon additives, a unique material being tested, compound degradation, or interactions between the extractables and other compounds or the formulation.

As part of the risk evaluation, it is often necessary to identify these compounds to gain a better picture of the possible health concerns. Unknown identification typically requires the use of multiple techniques, frequently in collaboration, to gain the necessary information on the compound. These techniques include, but are not limited to, high resolution analysis, MS/MS (or MSn) analysis, peak collection or purification, analysis using alternate chromatographic techniques and sample modification. Several case studies will be discussed to present real-world problems and solutions to unknown identification using GC-MS and LC-MS analysis.

<table>
<thead>
<tr>
<th>Keywords</th>
<th>Accelerated Solvent Extraction, Gas Chromatography/Mass Spectrometry, Liquid Chromatography/</th>
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<td>Application Code</td>
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Intravenous (IV) bags are widely used for drug delivery and other healthcare procedures. To ensure quality and consumer safety, extractable and leachable analyses must be carried out following regulatory requirements.

In this study, commercial IV bags extractables analyses were conducted following USP guideline. HR-LCMS, HS-GCMS, and GCMS analyses were carried out to identify volatile, semi-volatile, non-volatile compounds, and trace elements.

Methods

Commercial IV bags were extracted using pH 3 Water, pH 9 Water, IPA/Water (1:1), EtOH/Water (1:1), and Phosphate Buffered Saline (PBS). The bags were incubated at 50 °C for 24 hours. The extraction solutions were directly used for LCMS analysis.

LCMS analysis was carried out on a system consisting of Thermo Scientific Ultimate 3000 LC and Q Exactive Plus MS. Full scan MS and top 3 data-dependent HCD MS/MS data were collected with polarity switching. LCMS Data was processed using Thermo Scientific software Compound Discoverer 2.0.

The LCMS results show that pH 3 extract has one major extractable: DEHP, while pH 9 and PBS extracts have very similar profiles. IPA/Water (1:1) and EtOH/Water (1:1) extracts have similar but different profiles.

The High Resolution Accurate Mass (HRAM) full scan and MS/MS data acquisition with polarity switching proved to be very useful for extractable screening, which greatly assisted the identification and structure elucidation of extractables, especially for unknowns ID.

The HRAM full scan and ms/ms data was processed with Compound Discovererer 2.0 for known and unknown component identification and structure elucidation. The “Predicted Formula” based on accurate mass and isotope fidelity allow rapid database search and effectively reduced the false positives. It also assisted unknown component analysis by using the “Custom Explanations” feature.

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### Extractables and Leachables Analysis

#### Extractable Profiles of Packaging Materials for Permanently Implantable Medical Devices

Johnson & Johnson Medical Devices segment produces a broad range of innovative products used primarily by health care professionals. In the area of surgery, the segment offers leading surgical technologies and solutions including sutures, meshes, staplers, energy devices, trocars, and hemostats. These diverse product lines involve wide ranges of materials and processes. Various analytical techniques and critical in-house expertise are required to evaluate extractables/leachables to support new product development and product life cycle management.

Packaging materials for permanently implantable medical devices include various components, such as folders, trays, lids, and labels, which are made from a wide range of materials (foil, polymeric materials, coated paper, etc.). The packaging materials are also exposed to different sterilization processes including high dose gamma irradiation. Extractables/leachables of medical device packaging materials are important factors to consider as they could impact efficacy and safety of the final devices.

The major analytical challenges for profiling the extractables from medical device packaging materials are how to identify and quantitate the extractable compounds in the extracts. Two case studies related to chemical evaluation of the aqueous extractables from suture packaging materials will be presented. The first example is an unprinted paper label. It is used on the outside and/or inside of various suture product packages. The second example is about a low density polyethylene (LDPE) foam that is used for “parking” suture needles. The packaging components were extracted using deuterated water (D2O) for ease of analysis by NMR directly. The complete analytical characterization of the extractable profiles using various analytical techniques including NMR, MS, and IR will be discussed.

**Keywords:** Extraction, Identification, NMR, Polymers & Plastics

**Application Code:** Other

**Methodology Code:** Magnetic Resonance

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**Session #** 350  
**Abstract #** 350-6  
**Organized Contributed Sessions**

**Session Title**  
Extractables and Leachables Analysis

**Abstract Title**  
Extractable Profiles of Packaging Materials for Permanently Implantable Medical Devices

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Ethicon, Johnson & Johnson

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**Date:** Monday, March 06, 2017 - Morning  
**Time:** 10:25 AM  
**Room:** W184d
Extractables and Leachables Analysis

Extractables and Leachables from Single-Use Systems

With developments of bioprocessing equipment in the aspect of the range and scale of unit operations incorporating single-use technology, application of single-use systems in biopharmaceutical manufacturing has expanded considerably. Despite the many advantages of single-use systems such as flexibility and reduced costs, extractables and leachables are one of the major concerns to restrict the usage. Extractable studies are typically conducted using model solvent under worst-case conditions and reveal chemical entities that may migrate from process components into the final products as potential leachables. The identified potential leachable compounds from extractable study can facilitate the process- and product-specific extraction tests (leachables) with process fluid. A case study of extractables and leachables is reported in this presentation. Currently a systematic extractable study on each component from Pall single-use system according to BPOG standard protocol is ongoing.

Keywords: Liquid Chromatography/Mass Spectroscopy, Polymers & Plastics
Application Code: Polymers and Plastics
Methodology Code: Liquid Chromatography/Mass Spectrometry
### Extractables and Leachables Analysis

#### Ion Mobility-Mass Spectrometry: A Novel Approach to Screening for Extractable and Leachable Components from Packaging Material

Extractable and leachable components, which are potentially harmful to human health, are of great concern to manufacturing industries, particularly manufacturers of food contact materials, pharmaceutical packaging and devices as well as cosmetics packaging. Globally, much legislation exists to try to mitigate exposure to these components, which results in a significant demand for rapid, accurate, and reliable analytical methodologies. One such method is targeted screening using LC-MS techniques. 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 will vary. If the compound is present at trace levels, the fragments might be absent. In this work, we demonstrate how the inclusion of collisional cross section (CCS) values, acquired using ion mobility-mass spectrometry, can provide increased confidence in compound identification.

Collisional cross section is a key physicochemical property of compounds. The CCS depends on an ion’s size, shape and charge. For example, in the case of two ions with the same $m/z$ but different shapes, the less compact, straight-chain species will have a longer drift time than the smaller, more compact species.

**Abstract Text**

Extractable and leachable components, which are potentially harmful to human health, are of great concern to manufacturing industries, particularly manufacturers of food contact materials, pharmaceutical packaging and devices as well as cosmetics packaging. Globally, much legislation exists to try to mitigate exposure to these components, which results in a significant demand for rapid, accurate, and reliable analytical methodologies. One such method is targeted screening using LC-MS techniques. 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 will vary. If the compound is present at trace levels, the fragments might be absent. In this work, we demonstrate how the inclusion of collisional cross section (CCS) values, acquired using ion mobility-mass spectrometry, can provide increased confidence in compound identification.

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**Keywords:** Liquid Chromatography, Mass Spectrometry, Polymers & Plastics, Time of Flight MS

**Application Code:** Polymers and Plastics

**Methodology Code:** Liquid Chromatography/Mass Spectrometry
# Ionophore-Based Chemical Sensors II

## Fluorescence Nanosensor for Ratiometric Detection of Intracellular Calcium

Ionophore-based optical nanosensor (ION) has been widely employed to detect various ions in different environments. However, few have applied IONs in resolving real-time ion dynamics in living cells. To compare ION performance on tracing ion flux in intracellular space with its conventional ion indicator peers, we chose to monitor calcium transients, a well-understood process, by using ionophore-based nanosensors. The fluorescence intensity ratio obtained from two pH-sensitive dyes in the nanosensor provides a reliable optical metric of calcium levels. We first characterized the selectivity, reversibility, and dynamic sensing range of the nanosensor both in cell-free solution and intracellular environment, and then compared the intracellular calcium dynamics obtained from nanosensors with calcium dye indicators during pharmacological stimulation. This study could help better understand the ability of ionophore-based nanosensor to probe intracellular ion dynamics, and opens up new opportunities for live cell monitoring of other ion targets such as sodium and chloride due to its modular design and ease of preparation.

**Keywords:** Imaging, Ion Selective Electrodes, Nanotechnology, Sensors

**Application Code:** Nanotechnology

**Methodology Code:** Sensors
Adsorption of an ionophore, ion-exchanger and chromoionophore on cellulose paper has been shown to enable heterogeneous optical ion selective sensing in the absence of any plasticizer (Chem. Commun. 2015, 51, 15176). Such sensing platform has been found to be universal for colorimetric analysis of cations including Na\(^{+}\), K\(^{+}\), Ca\(^{2+}\), Ag\(^{+}\), and Pb\(^{2+}\). Herein, we designed the first paper-based plasticizer-free fluoride (F\(^{-}\)) selective optode and examined its application in F\(^{-}\) sensing in drinking water. The F\(^{-}\) optode was fabricated by inkjet printing of aluminum(III)-octaethylporphyrin and ETH 7075 dissolved in cyclohexanone onto filter paper. A camera phone was used to record the color of the optode in the presence of different concentrations of F\(^{-}\) and the hue of the optical sensing coating on the paper was used for quantification. In glycine-phosphate buffer at pH 3.0, F\(^{-}\) levels as low as 10\(^{-5}\) M are readily detectable by this method and the response time is within 15 min. The selectivity over anions such as chloride, phosphate, sulfate, perchlorate, thiocyanate, chlorate, and bromate meets the selectivity requirement for measurement of F\(^{-}\) concentration in municipal drinking water. To enable direct analysis of F\(^{-}\) in drinking water, a mildly acidic buffer was printed on paper in an area adjacent to the optode sensing area and the whole paper was sealed in a laminating pouch to prevent sample evaporation. The water sample is wicked through a hole in the pouch into the buffering area and then into the optode area to induce the color change. Since existing commercial F\(^{-}\) test strips require pretreatment of the sample with concentrated hydrochloric acid and suffer from interference from sulfate, chlorate, and bromate, the proposed technique may allow more facile and accurate analysis of F\(^{-}\) in drinking water.

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

**Application Code:** Environmental

**Methodology Code:** Sensors
Light and electrodes have a long history with spectroelectrochemistry, electrochemiluminescence and photovoltaics. Here we exploit light shined on an electrode to locally activate the electrode surface to allow faradaic electrochemistry to occur on the illuminated spot only. The light is shone onto a self-assembled monolayer modified silicon electrode where faradaic electrochemistry is observed at defined locations whereas in the dark no electrochemistry is observed. To achieve this, first an oxide free silicon electrode is modified with a self-assembled monolayer of 1,8-nonadiyne which protects the surface against oxidation. Coupled to the monolayer is an electrochemical cleavable unit and to that unit antibodies to capture specific cell types. Upon cell adhesion, the response of the cells to drugs or other soluble cues, can be monitored either microscopically or using impedance spectroscopy. Cells that show unusual behavior, can then be released one cell at a time, for further analysis. This is achieved via the application of a sufficiently reducing potential and illumination of just that cell. The methodology has utility for both fundamental understanding of cell heterogeneity and/or precision medicine.

Keywords: Biosensors, Electrochemistry, Sensors
Application Code: Biomedical
Methodology Code: Electrochemistry
The synthesis of upconverting particles of NaLuF$_4$ is reported in high yield. With their capacity for excitation in the NIR and emission in the visible, they are potentially useful materials for in vivo and in situ use. Upconversion is more efficient than two-photon absorption because it takes advantage of the long life-time of the excited energy states. Surface modification allows for a wealth of chemistries to be introduced that can provide selectivity towards interaction with different analytes.

The synthesised particle gives both green and red emission at 536nm and 646nm respectively. The green emissions result from the $[^2H_{11/2}]$ and $[^4S_{3/2}]$ to $[^4I_{15/2}]$ transitions, respectively, and the red emission originates from the $[^4F_{9/2}]$ to $[^4I_{15/2}]$ transition.

The existence of two emissions offers a unique opportunity to build in a reference signal and this presentation demonstrates how that can be used to provide selective ion sensitivity. The preliminary demonstration of pH is also demonstrated for different particle sizes. The capacity to synthesise particles that are small enough for straightforward measurements within the cell is explored.

Abstract Text
Microfluidic paper-based analytical devices (microPADs) have gained a lot of attention due to their potential to become user-friendly, disposable and low-cost alternatives to more instrument-intensive analytical approaches. Liquid sample flow driven by capillary forces along patterned flow channels through the microporous structure of paper is one point common to most microPADs.

Due to the physical-chemical properties of cellulosic paper, in particular its relatively large surface-to-volume ratio and the presence of chemically functional groups, interactions between the analyte and the paper matrix have a significant influence on the analytical results. What is an important feature used in paper chromatography for separation purposes can become an unwanted side-effect hindering efficient analyte transport and sensitive analysis on microPADs. For example, the presence of carboxylic acid residues originating from the paper-making process can significantly reduce the transport of multivalent cations in paper-based microfluidic channels. Such negative effect is enhanced by increasing lengths of flow channels. Furthermore, the cation-exchange properties of paper can no longer be neglected when working with ionophore-based sensing mechanisms on microPADs.

While the transport of aqueous liquids through porous fibrous materials of microPADs has been intensely studied, information on the effective amounts of transported analyte is still scarce, although the “chromatographic effect” of the paper matrix has been known for decades. In this study, various parameters influencing the analyte transport and the analytical performance of microPADs based on colorimetric signal detection have been investigated both qualitatively and quantitatively. Among others, it is demonstrated that the transport of the effective amount of an analyte on a microPAD can significantly differ from the transport of the sample liquid that the target analyte is dissolved in, and that these differences are strongly dependent on the type of analyte. Awareness of these factors and their quantification is expected to contribute to sensitivity improvements of microPADs.

Keywords: Lab-on-a-Chip/Microfluidics, Sensors
Application Code: Biomedical
Methodology Code: Sensors
The high ion selectivity of potentiometric and optical sensors based on ionophore-based polymeric membranes is thermodynamically limited. Here, we report that the voltammetric selectivity of thin ionophore-based polymeric membranes can be kinetically improved by several orders of magnitude in comparison with their thermodynamic selectivity. The kinetic improvement of voltammetric selectivity is evaluated quantitatively by newly introducing a voltammetric selectivity coefficient in addition to a thermodynamic selectivity coefficient. Mechanistically, we propose a new hypothetical model that the slower transfer of a more hydrophilic ion is controlled by its partial dehydration during the formation of the adduct with a “water finger” prior to complexation with an ionophore at the membrane/water interface.
Solid state potentiometric sensors have an inherent advantage of miniaturization. We developed a carbon based solid state Ion Selective Electrode (ISE) sensors. This unique low PVC content sensor is capable of performing amperometric measurements and then could be switched to potentiometric measurements. The 35-40 [micro]m diameter Ca2+ [micro]-ISE showed fast response time (~1 sec or less), low limit of detection (~1 [micro]M) and broad linear range (5 [micro]M to 200 mM). In addition, the Ca2+-[micro]-ISE was proven to be insensitive towards redox molecules while demonstrating excellent selectivity towards major interfering ions (such as Na+, K+, and Mg2+, with logKCa2+,A = -5.5 to -6.0). Similarly, carbon based [micro]-pH sensor has also been developed which showed Nernstian slope and broad linear range of 4.5 to 10. Recent characterization of this unique carbon based sensing membrane will be presented in the conference.

This work was supported by National Institute of Dental and Craniofacial Research (NIDCR), NIH (Grant # R21DE025370).

Keywords: Bioanalytical, Biosensors, Electrochemistry, Ion Selective Electrodes
Application Code: Bioanalytical
Methodology Code: Electrochemistry
The Nano-Interface between Two Immiscible Electrolyte Solutions (ITIES) provides a unique analytical platform for the study of environmentally and biologically relevant ionic species, such as heavy metal ions and neurotransmitters. A typical nano-ITIES consists of a laser pulled quartz pipette with an orifice in the nm that can be filled with an immiscible organic solution, where an ionophore can be added to modulate the ionic species detection via a scheme called assisted ion transfer. Upon electrochemical polarization, charged ionic species can be transferred from one phase to another, which is the basis for quantitative ionic species sensing. This is particularly useful for the detection of non-redox active neurotransmitters, especially those whose detection on carbon microelectrodes is challenging, such as Acetylcholine, whose detection on the carbon microelectrodes often requires enzymatic modification.

In this communication, we studied on the nanopipette based sensor probe the detection of environmentally relevant metal ions and neurotransmitters; a new ionophore with multiple binding sites, TriBCE, was utilized for the assisted ion transfer study of these ions. TriBCE contains three 18-Crown-6 moieties connecting to a common center. Comparative studies for the above mentioned ionic species detected with nanopipet electrodes were made via assisted ion transfer by DB18C6 and TriBCE ionophores. The strategy presented here is essential for the understanding of assisted ion transfer and the experimental findings can provide guidance for developing ionophores related to ITIES sensor probes.

Acknowledgement: This research was supported by the National Institutes of Health under Award Number R21NS085665.
Plasma based ambient desorption/ionization (ADI) sources generally use helium as the discharge gas due to the large reaction cross section of excited helium species with atmospheric gases, which leads to large reagent-ion densities. While most plasma-based ADI sources utilize a pure discharge gas, here, we explore changes in discharge processes and ionization chemistry with the addition of various molecular gases to the helium discharge gas of a flowing atmospheric pressure afterglow (FAPA) source. It was found that the abundance of protonated water clusters increased by five times with the addition of 0.1% v/v oxygen to the helium discharge gas. Correspondingly, an increase in protonated analyte signal was also observed for small polar analytes, such as methanol and acetone. However, when analytes containing aromatic constituents were desorbed/ionized with a He:O2 FAPA, (M+3)+ ions were detected, while no molecular or protonated molecular ions were observed. These (M+3)+ ions were characterized through exact mass measurements revealing their identity as pyrylium ions formed through substitution in an aromatic ring. The presence of pyrylium-based ions was further confirmed by tandem MS of the (M+3)+ ion of 1,3,5 trimethylbenzene compared to that of a commercially available 2,4,6 trimethylpyrylium salt. Furthermore, rapid and efficient production of pyrylium in the gas phase was used to convert benzene into pyridine. In another case, hydrogen addition drastically decreased reagent-ion population; however no corresponding decrease in analyte ion signal was observed under these conditions. Interestingly, molecular mass spectra produced from He:H2 FAPA showed a significant change in ionization pathway. The potential ionization mechanisms with mixed-gas FAPA will also be presented.

Keywords: Chemical Ionization MS, Instrumentation, Organic Mass Spectrometry, Plasma
Ion sources for molecular mass spectrometry (MS) are usually driven by direct current (DC) power supplies with no user control on the generated charges. Such control over ion generation would bring a new parameter for MS, as well as new capabilities for experimental design. Here, we present the first rational approach to quantitatively control the number of ions generated for MS analysis using triboelectric nanogenerators (TENGs). Quantized charge generation was achieved by using TENGs, which have fixed charge density. The voltage of this quantized charge is sufficient to induce nanoelectrospray ionization (nanoESI). For a given nanoESI emitter, accurately controlled ion pulses ranging from 1.0 to 5.5 nC were delivered, with an onset charge amount measured at 1.0 nC. The flexibility of generating single polarity or alternating polarity ion pulses was also demonstrated. Spray pulse duration control was demonstrated to be between 60 ms and 5.5 s. The effective voltage for the quantized charge delivered was adjusted as high as 9 kV. This super-high voltage electrospray did not cause observable corona discharge that would typically damage the nanospray tip. Instead, highly sensitive (~0.6 zeptomole) MS analysis with minimum sample consumption (18 pL pulse-1) was achieved. Experiments also showed that native protein conformation can be conserved with this approach. Patterned ion deposition onto both conductive and insulating surfaces was also demonstrated. With increasing charge supplied, plasma discharge ionization was also attained. This simple, safe, and effective TENG-driven ion generation approach opens the possibility of charge-resolved mass spectrometric analysis and ion utilization.

Keywords: Electrospray, Organic Mass Spectrometry, Sample Introduction
Application Code: General Interest
Methodology Code: Mass Spectrometry
Nanoparticle (NP) production and application are surging. This revolutionary advancement is benefiting many fields while also causing serious concerns of adverse impacts to human health and ecosystem. The development of analytical methods for NP characterization and quantification is one of the most urgent priorities for nanotechnology research and development, and is a major challenge in the environment (very low concentrations) and biological system (highly complex matrix). Advances in understanding impacts of NPs can only be made when detection, quantification, and characterization methods are developed.

In response, we have recently developed several robust sensitive and high-throughput single particle inductively coupled plasma-mass spectrometry (SP-ICP-MS) methodologies for metallic element based NPs analysis. The NPs include some of the most broadly used nanoparticles: silver (Ag), gold (Au), titanium dioxide (TiO2), cerium dioxide (CeO2), and zinc oxide (ZnO). A PerkinElmer NexION 350D ICP-MS with Syngistix™ (Nano Application module) was used for these analyses. This is the only SP-ICP-MS software on the market dedicated for nano material analysis. These methods can detect particle size, size distribution, particle concentration, and dissolved metal element concentration simultaneously. Utilizing the SP-ICP-MS methods enabled the study of the fate, transportation, and transformation of the NPs in agricultural crops, surface and drinking water systems, and commercial sunscreens. These emerging SP-ICP-MS methodologies will play critical roles and constitute a significant part of the risk assessment of engineered NPs in environment, human health, and ecosystem.

The projects were supported by Missouri Department of Natural Resources, PerkinElmer, Inc., and University of Missouri Research Board.

Keywords: Environmental Analysis, ICP-MS, Method Development, Nanotechnology
Application Code: Nanotechnology
Methodology Code: Mass Spectrometry
A laser ionisation mass spectrometry is thriving in Manchester. We have 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 analysis of proteins and femtosecond chemistry.

The developed technology incorporates laser desorption/ heating and cryogenic sample preconcentration, a range of tunable pulsed (ns/ fs) lasers coupled with the ion sources of time of flight, magnetic sector or accelerator beamline facilities. The resonance ionisation mass spectrometry is ideal 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: Isotope Ratio MS, Laser, Time of Flight MS, Ultratrace Analysis
Application Code: Nuclear
Methodology Code: Mass Spectrometry
Advances in Mass Spectrometry

Open Probe Fast GC-MS - Real Time Mass Spectrometry Analysis via Ambient Desorption, Ultra-Fast Separation and In-Vacuum Ionization

Ambient desorption ionization techniques such as DART, DESI, DSA, ASAP and LDTD are gaining attention and growing use. They enable real time sample on surfaces analysis, without sample preparation, while using the MS of LC-MS systems. However, they do not share the library identification strength of EI and the obtained mass spectra are often too complex hence require expensive High resolution Mass spectrometers.

We developed an Open Probe Fast GC-MS for real time analysis with separation, without sample preparation and with MS of standard GC-MS. It is based on a heated oven that is mounted on a low thermal mass fast GC which is open to room air with helium flow protection to prevent air penetration. Its operation is as simple as: touch the sample, insert it and get the GC-MS data in 30 s.

Several Open Probe Fast GC-MS forensic and other application were explored including: a) Synthetic cannabis on tobacco leaves; b) Cannabis flowers analysis for their content. c) Street drug heroin powder was analyzed in 30 s with its main 6 ingredients separated and identified; d) Trace TNT on human hands. e) Pesticides were analyzed in 40 s on tomato that was sprayed with cockroach killer.

Open Probe Fast GC-MS provides:
A) Library based sample identification with names and structures
B) Ultra-fast GC separation for improved mixtures analysis
C) Uniform response
D) Non-polar compounds are amenable for analysis
E) Swabs can be used to bring samples from remote surfaces
F) Low cost MS of GC-MS is used

Keywords: Forensics, Gas Chromatography/Mass Spectrometry, Mass Spectrometry
Application Code: Homeland Security/Forensics
Methodology Code: Mass Spectrometry
The contamination of foods with undesired chemical molecules – sometimes odorous in nature – can arise from varying sources via diverse routes. Mineral oil residues in processing machinery or in packaging materials, for example, may lead to unwanted contaminants such as mineral oil hydrocarbons (MOH) in foods. In many cases the detection of the presence of such contaminants would be ideally achieved using on-line analytical screening tools. When sensorially-active by-products or contaminants are present – originating from packaging materials, pigments or additives and leading to sensory defects – these might not be detectable by conventional means when they are present at ultra-trace concentrations yet have high odour potency. Identification of these physiologically or sensorially critical substances currently requires an arsenal of often complex and costly analytical tools such as gas chromatography-mass spectrometry (GC-MS), or two-dimensional high-resolution GC-MS, coupled with olfactometry, which must be operated by expert sniffers for directly targeting the odorous contaminants.

There is thus an increasing demand for fast analytical tools that allow high-throughput monitoring of food and packaging to screen for unwanted substances. Chemical ionisation-based techniques such as proton-transfer-reaction mass spectrometry offer a potential solution, enabling real-time detection of gas-phase volatile organic compounds (VOCs) down to sub-pptv concentrations. The recent use of a time-of-flight mass spectrometer (PTR-TOFMS) has improved the spectral resolution by three orders of magnitude whilst maintaining instrumental sensitivity and detection limits. Further, PTR-TOFMS offers the ability to scan an entire mass spectrum at several Hertz frequency, thereby providing the key parameters required for a real-time screening tool. The potentials and limitations on the detection of volatile contaminant analysis in foods will be addressed in this lecture.

Keywords: Food Safety, Headspace, Mass Spectrometry, Trace Analysis
Application Code: Food Safety
Methodology Code: Mass Spectrometry
The solution cathode glow discharge (SCGD) is an atmospheric pressure glow discharge that was designed as a source for atomic emission spectroscopy (AES) and, in that mode of operation, has offered detection limits comparable to inductively couple plasma AES. The SCGD consists of a direct-current plasma sustained between a metal anode and a flowing analyte-containing liquid, which serves as the cathode. Solution-phase analytes are sputtered into the discharge where they are excited and/or ionized. Though the SCGD is capable of generating ions as well as excited species directly from a sample solution, it had been used exclusively as a source for optical spectroscopy. Here, we describe the use of the SCGD as an ionization source for mass spectrometry. Elemental ion signals were obtained for a variety of inorganic salts with detection limits in the sub-part-per-billion range. The SCGD was found to be useful as a molecular ionization source where samples could be introduced into the flowing cathode solution or by holding a solid sample in close proximity to the discharge akin to an ambient desorption/ionization experiment. Lastly, it was found that the SCGD was capable of forming gas-phase ions of solution-phase, large biomolecules, such as peptides. Furthermore, it was found that peptides could be tunably fragmented through simple adjustment of the source power. Analytical performance of SCGD-MS for these different analyte classes will be presented as well as a discussion of potential ionization and fragmentation mechanisms.
Mercury is a known biological toxin that causes devastating morbidity and mortality when humans are exposed to the several common ionic and molecular species. Mercury exists in several common forms in the environment and each species has different toxicological consequences for adults and for unborn children. The most common two species are organic (usually methyl mercury) and inorganic mercury (usually doubly charged). Organic mercury species are especially hazardous to human health due to the ability to cross the blood-brain and placental barrier. Previously, agencies such as the California Department of Toxic Substances Control (DTSC) and the Environmental Protection Agency (EPA) have been monitoring the levels of total mercury in drinking water. Because toxicity and risk factors differ greatly depending on species, measurements of the metals’ species are being proposed in legislation. California would like to establish lower allowable concentration of methyl mercury in drinking water to protect their populations, including pregnant mothers and their unborn children. These populations are highly susceptible to exposure of methyl mercury due to the risk of Minamata disease damaging the fetus. California would like to reduce the lower limit of quantification of inorganic and organic mercury to 0.2ppt and 0.02ppt respectively. This study is the research and development of an automated IC instrument for the pre-concentration of drinking water mercury species, and couples with an ICP-MS using EPA method 6800 for quantification of each species. Method 6800 allows for the correction of species conversion that can occur during the analysis enabling legally defensible and actionable monitoring.

Keywords: Environmental/Water, Ion Chromatography, Mass Spectrometry, Ultratrace Analysis
Application Code: Environmental
Methodology Code: Mass Spectrometry
Exosomes are emerging as powerful biomarkers for the diagnosis and monitoring of cancer. However, the translation of exosome diagnostics to the improvement of patient care has been limited by the inability of existing technology to isolate and detect specific populations of these nanoscale vesicles for clinical testing. Due to their small sizes (30 nm - 200 nm), conventional isolation of exosomes consists of time consuming (> 12 hr) ultracentrifugation and results in co-purification of cell debris and microvesicles. To address this challenge, we report a new architecture, wherein millions of nanofluidic devices are incorporated onto a single monolithic platform, increasing throughput by a million fold and thus making use in diagnostics possible. Our track-etched magnetic nanopore(TENPO) device consists of an ion track-etched polycarbonate membrane with 600 nm diameter pores coated with a soft magnetic film, permalloy (Ni20Fe80). This design rotates the conventional microfluidic - immunomagnetic sorting structure by 90 degrees to form magnetic traps at the edges of pores instead of in channels. (Fig. a) Using TENPO we isolated exosome sub-populations based on either expression of pan-exosome surface markers(CD81, CD63, CD9) or known cancer epitopes(EpCAM, Intb1, Muc1). (Fig. b) Isolation is performed directly on V =10 mL clinical serum and plasma samples, without clogging, in less than 30 minutes. Moreover, because the entire assay is incorporated onto a monolithic platform, recovery of exosomal RNA and DNA was achieved with greater yield (> 3x improvement) and purity (> 90%) than conventional methods. (Fig. c, d) To demonstrate the power of this technique, we extracted exosomal RNA and DNA biomarkers for the early diagnosis of pancreatic cancer directly from serum samples, and established its potential clinical efficacy using an in-vivo murine model and pilot clinical samples. (Fig. e)
Cancer treatment currently focuses on radio-, chemo- and immune-therapeutic techniques; however, the toxicity of these techniques toward normal cells results in numerous side-effects. Thus, it is essential to develop new techniques that show selectivity toward cancer cells in order to reduce these side effects. In this regard, nanomedicine has been widely investigated for targeted chemotherapeutic applications. Recently, Rhodamine 6G nanoparticles, nanoGUMBOS, synthesized from a Group of Organic Materials Based on Organic Salts (GUMBOS), have been found to express selective toxicity toward cancer cells. The studies presented here focus on using cyclodextrin to enhance the toxicity of these nanoGUMBOS toward breast cancer cells. Cyclodextrin has been widely used for drug delivery applications in order to enhance stability of the drugs in aqueous medium; thus, use of organic solvents to solubilize these drugs is eliminated. For studies reported in this presentation, nanoGUMBOS were synthesized using an ion-association method. Transmission Electron Microscopy (TEM) was used to characterize the nanomaterials formed. A reduction in size was observed for nanoGUMBOS formed in the presence of cyclodextrin. Following TEM characterization of these nanoGUMBOS, these nanomaterials were tested for toxicity toward breast carcinoma cells. Intriguingly, a three to four fold increase in the toxicity of the nanoGUMBOS was observed with nanoparticles formed in the presence of cyclodextrin. In addition, these nanoGUMBOS remained non-toxic to normal cells, indicating that the cyclodextrin did not affect selectivity. Moreover, these studies gave further insight into potential use of these nanoGUMBOS as chemotherapeutics.

Keywords: Biopharmaceutical, Cyclodextrin, Drug Discovery, Nanotechnology
Application Code: Biomedical
Methodology Code: Biospectroscopy
Nanotechnology for cancer applications has undergone significant advances over the past few decades. Numerous nanoparticle-based products have been approved for clinical practice and many more are under clinical trials. However, most of the current studies are focused on the therapeutic applications rather than developing novel methods to study, diagnose and guide the therapy of cancer, such as monitoring tumor pH. In here, we encapsulated a commercially available pH indicator dye, SNARF-5F, into surface modified polyacrylamide nanoparticles. The nanoparticle encapsulation serves not only as a targeted delivery tool but also to protect the SNARF-5F from interference by body proteins or enzymes. Such interference is known to cause changes in the dye’s optical properties, thus affecting the pH calibration. Utilizing the non-invasive and deeply penetrating technique of photoacoustic (PA) imaging, we hereby propose and present a method for obtaining cross-sectional pH images of tumors in vivo. There are a few recently reported works on PA pH imaging in vivo; however, they are unable to provide quantitative pH images, because of background signal interference due to optical absorption by the tissues. We remove from our images the background signal from the most absorbing factor in biological tissues, namely, “blood”, which allows us to directly provide pH images. The average pH of the tumors is found to be 6.61 ± 0.34, while the average pH of regular tissue is 7.46 ± 0.1. These results match “gold standard” pH electrode measurements done ex vivo. Thus, this approach provides us with the first demonstration of optically based quantitative pH images taken on in vivo tumor models.
The development of effective screening methods for early cancer detection is one of the foremost challenges facing modern cancer research. Urinary metabolomics has recently emerged as a potentially transformative approach to cancer biomarker discovery owing to its noninvasive sampling characteristics and robust analytical feasibility. As an extension of conventional metabolomics, urinary metabolomics has benefitted from recent technological developments in nuclear magnetic resonance, mass spectrometry, gas and liquid chromatography, and capillary electrophoresis that have improved urine metabolome coverage and analytical reproducibility. Meanwhile, extensive metabolic profiling in urine has revealed a significant number of altered metabolic pathways and putative biomarkers, including pteridines, modified nucleosides, and acylcarnitines, that have been associated with cancer development and progression. In this talk, we will provide an overview of new developments in urinary metabolomics by covering the most promising aspects of hyphenated techniques in untargeted and targeted metabolomics, followed by a meta-analysis of recent untargeted studies from which we have compiled an extensive list of putative biomarkers that have been revealed by urinary metabolomics. Finally, we will discuss the technical and clinical limitations as well as emerging challenges in the field of urinary metabolomics and its application to cancer biomarker discovery, such as the biological variation of urinary metabolomics and methods to adjust urinary metabolite levels to urine concentration-dilution, and offer future perspectives for how the field is anticipated to change over the next decade.

**Keywords:** Biomedical, Capillary Electrophoresis, Gas Chromatography/Mass Spectrometry, Liquid Chromatography

**Application Code:** Biomedical

**Methodology Code:** Education/Teaching
Biomedical - Novel Techniques

Abstract Title
Single Cell ICP-MS Quantification of Metal Content in Individual Cells - An Insight into Cancer Treatment

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Abstract Text
Cisplatin, carboplatin, and oxaliplatin are the most widely used class of anticancer agents used to treat many types of cancer. The mechanism of action for platinum compounds is DNA damage resulting in cell death. Initially, patients respond well to treatment but later relapse and display resistance to platinum compounds. Resistance to platinum compounds is mediated by the following mechanisms: decreased drug uptake, increase drug export, increased DNA repair, and cytosolic inactivation. This work focuses on the use of Single Cell Inductively Coupled Plasma-Mass Spectrometry (SC-ICP-MS) technique exploring the uptake mechanism for cisplatin in cells based on individual cells. Experiments were performed using the A2780 cisplatin-sensitive ovarian carcinoma cell line and the corresponding cisplatin-resistant cell line, A2780-CP70. Cells were treated with 3 µM of cisplatin and uptake was analyzed by a time course experiment 1, 2, 4, and 8 hours post-cisplatin exposure. Total cellular cisplatin was measured analyzing the platinum 195 isotope using the Syngistix Application Single Cell Module. The Syngistix Single Cell Module allows the determination of platinum within each cell and creates a histogram of cisplatin uptake. The uptake of cisplatin differed between the cisplatin-sensitive A2780 cell line in comparison to the A2780-CP70 cisplatin resistant cell line. Additionally, we observed a heterogeneous distribution of cisplatin uptake in both cell lines, reflecting that drug uptake within cancer cells differs from cell to cell. Single cell analysis allows the real time uptake of cells, reflecting more of what is observed within tumor yielding the possibility to determine resistance status. Additionally, SC-ICP-MS allows for the development of experimental models to determine drug delivery and efficacy translating to a better response in the clinic.

Keywords: Biological Samples, Biopharmaceutical, Drug Discovery, ICP-MS
Application Code: Biomedical
Methodology Code: Atomic Spectroscopy/Elemental Analysis
The emerging field of diagnostics for detecting protein biomarkers faces certain challenges like low abundance in human samples, lack of sensitivity and binding capacity, poor reproducibility, large sample volume requirement, high cost etc. To address these technical aspects for clinical validation, a highly sensitive, selective, rapid and compact immunoassay system is under development which performs testing with few nL of human serum and targets low abundance proteins efficiently. The procedure includes elimination of albumin, globulins and other undesirable species using affinity driven segregation for highly specific immunoreaction and separation of these target molecules using iso-electric focusing method in PDMS-glass devices.

Bound/free separation is performed in nano/micro depth channels where the immunocomplex formation is analyzed using fluorescence detection. In the procedure, a pH gradient is established using carrier ampholytes and 0.5% methyl cellulose is surface coated for suppressing electro-osmotic flow and reducing non-specific adsorption. Separate sharp bands of fluorescent molecules validating the assay reaction mechanism are obtained on voltage application. The focusing is observed in <10 seconds with a voltage requirement of only 1-2.5V in 700nm depth channels. A concentration factor of around 100 is also achieved as a result of the high resolution fractionation.

Prior to the separation, removal of high abundance undesirable proteins such as albumin and globulins is carried out using affinity labels for target molecule capture and washing out remaining solution. The sample is then fed into the nano/microchannels and concentrated for semi quantitative analysis.

The high performance low-cost assay system incorporating nanoscale phenomena and corresponding technical and commercial benefits deems fit for the purpose of primary care, providing rapid and targeted information by identifying specific biomarkers for deeper understanding of the disease and patient heterogeneity, especially in developing countries.
Online PTR-ToF-MS Applications Reveal the Influence of Oral and Nasal Routes of Breathing on Exhaled VOC Profiles

Breath VOC patterns have been proposed as future diagnostic tool. As breath can be sampled via oral or nasal route, we applied real-time mass spectrometry to investigate sampling related effects on exhaled VOC concentrations.

An online PTR-ToF-MS-8000 [PDrift=2.3mbar, TDrift=75°C, VDrift=610Volt, E/N=139Td, Time-resolution=200ms] was applied for breath-resolved VOC profiling in 15 healthy subjects, in continuous side-stream mode [sampling flow=20sscm] during paced breathing (12/min) for 8 min. Breathing route was switched after every 2 minutes; i.e. oral → nasal → oral → nasal. VOC concentrations were assigned to alveolar and inspiratory phases by MATLAB algorithm. Continuous spirometry, capnometry and hemodynamic monitoring were performed in parallel.

PET-CO2 and normalised mean alveolar concentrations of endogenous isoprene decreased significantly by 6% and 13% during initial nasal breathing, thereby inversely mirroring the increase of tidal-volume and minute-ventilation by 15% and 16%, respectively. Endogenous acetone and C2H6S remained constant. During nasal breathing, H2S (oral origin) and C4H8S (dietary origin) decreased by 28% and 14% respectively. These changes were reproducible in the second part of the experiment. Smoking related compounds e.g. acetonitrile and furan and exogenous benzene and toluene also remained consistent. Cardiac output did not change significantly.

Switching of breathing routes instantly changed exhaled VOC concentrations. Such substance specific effects mainly depend on respiratory parameters, on potential origin and physico-chemical properties of the compounds. Reliable sampling must define any particular breathing route before interpreting changes in exhaled VOC concentrations as disease biomarkers.

Keywords: Bioanalytical, Biomedical, Mass Spectrometry, Sampling
Application Code: Biomedical
Methodology Code: Mass Spectrometry
The techniques currently used for drugs, metabolites and biomarkers determination are based on sample collection, and therefore they are not suitable for repeated analysis because of the high invasiveness. Here we present a novel method of biochemical analysis directly in organ/tissue without a need of sample collection and/or homogenization step: solid phase microextraction (SPME). The approach is based on flexible microprobe coated with biocompatible extraction phase, which is inserted to the tissue with no damage or disturbance of the organ/biopsy tissue. The technology allows assessment of organ function by biochemical profiling, determination of potential biomarkers and drug monitoring when SPME is coupled to LC-MS or directly MS. Up-to-date it was demonstrated during lung and liver transplantation, brain function monitoring during deep brain stimulation and drug administration, in vivo lung perfusion for local chemotherapy, and more recently, brain tumor metabolomic study. In the current study we used animal models to assess a metabolic changes in kidney during organ preservation for several hours. The data showed significant changes in compounds associated with glutathione, amino acids and purine metabolism as a result of oxidative stress and ischemia. We have also performed spatial resolution studies comparing metabolic profile of cortex and medulla demonstrating possibility of minimum invasive sampling of target areas located inside the organ.

**Keywords:** Biomedical, Sampling, Metabolomics, Metabonomics

**Application Code:** Biomedical

**Methodology Code:** Liquid Chromatography/Mass Spectrometry
Holographic video microscopy is used to characterize model wastewater samples containing multiple different types of contaminants. Traditional light scattering techniques can measure the distribution of particle sizes, but are unable to distinguish different species of sub-visible particles present in the same sample. In combination with Lorenz–Mie theory, holographic video microscopy can determine the size, refractive index, and three-dimensional position of each particle in a suspension with high precision and accuracy. An individual particle’s refractive index offers insights into its composition that cannot be obtained with any other particle characterization technique. This refractive index measurement capability allows differentiation of oil emulsion droplets from polystyrene microbeads, bacteria and other co-dispersed materials as demonstrated in our results. Finally, we demonstrate that this approach is fast enough to rapidly build up population data and to simultaneously track concentration changes in specific contaminants, such as micrometer-scale suspended solids and emulsions.
Due to the widespread use of pharmaceuticals in both human and animal populations, the contamination of surface waters resulting from the outflow of water treatment facilities is of growing concern. Conventional analysis methods often require extensive sample preparation in order to achieve appropriate limits of detections and quantitation. As a result, analytical methods which utilize these procedures are limited in their throughput capacity, while also generating large volumes of solvent waste. Coated blade spray (CBS) is a SPME technique which enables the direct to mass spectrometry analysis of extracted compounds with the application of limited organic solvent in order to desorb analyte and perform electrospray ionization. Demonstrated herein is the application of CBS for the concomitant MS/MS analysis of 12 pharmaceuticals in environmental waters. In the study described, a hydrophilic-lipophilic balanced sorbent phase was used, as the target compounds chosen represented a wide variety of physical chemical properties. Parameters such as pH of sample, extraction time and desorption/spray solution composition were investigated. Samplings as short as 2 minutes resulted in the detection of all compounds in the low ppt range while demonstrating LOQ values in the 10s of ppts for some compounds. Certainly, the application of CBS for the analysis of pharmaceuticals in surface waters has the potential to provide analytical scientists with a tool to perform rapid, and relatively solvent free sample analysis as an alternative to conventional analytical methods for both screening and quantitative purposes.
A topic of growing importance to environmental scientists is the presence of contaminants of emerging concern (CECs) in municipal wastewater treatment facilities and the possibility of them remaining post-treatment. Many of the current treatment technologies are not effective at removal of these CECs. In this study, CECs from personal care products, industrial processes, and pharmaceuticals were analyzed. Pre-treatment influent, post-treatment effluent, and irrigation water samples from post wastewater treatment were extracted and analyzed by multidimensional gas chromatography (GCxGC) coupled to time of flight mass spectrometry (TOFMS). Liquid-liquid extraction (LLE) and polydimethylsiloxane stir bar sorptive extraction (SBSE) were compared for their extraction efficiency, “greenness”, and applicability. The data from this project finds CECs present in the influent, effluent and spray water signifying that many are not being removed during the treatment process. Both extraction methods were effective, LLE extracts a more broad range of compounds but SBSE is more sensitive. SBSE is a much “greener” and more facile approach than LLE. GCxGC TOFMS allows for excellent separation of these complex mixtures and is especially useful in the identification of the CECs in wastewater samples. Finally, the use of software generated reference methods, calibrations, exclusion zones and classifications will be discussed to allow for greater ease of data processing and reduction.

Keywords: Capillary GC, Environmental Analysis, Environmental/Water, Time of Flight MS
Application Code: Environmental
Methodology Code: Gas Chromatography/Mass Spectrometry
Environmental Analysis of Water Quality

Determination of Trace Concentrations of Oxyhalides and Bromide in Municipal and Bottled Waters Using a Compact Ion Chromatography System

Water disinfectants can react with naturally occurring materials in the water to form unintended disinfection byproducts such as oxyhalides. Bromate has been identified as an animal carcinogen and potential human carcinogen. Careful monitoring of bromate is required to ensure it does not exceed safe drinking water standards. This study demonstrated that oxyhalides and bromide can be determined accurately in municipal drinking water and bottled water using 4 µm anion-exchange column and high pressure ion chromatography system with suppressed conductivity detector. A short 25 min gradient method was developed in this study. The eluent was 10 mM KOH for 10 min and then increased to 30 mM from 10-18 min; finally keep at 100 mM from 18-25 min to elute the remaining inorganic anions. In addition to shortening analysis time, this method delivered detection limits for chlorite, bromate, chlorate, and bromide that were all below 0.5 ppb. The low detection limits resulted from the excellent peak efficiency of the 4 µm column combined with low noise and exceptionally low suppressed background conductivities obtained using a thermally regulated environment detector compartment. All four anions demonstrated accepted recovery (90-110 %) according to the criteria outline in U.S. EPA Method 300.1 Part B. In addition, using a reagent free ion chromatography system with electrolytically generate potassium hydroxide eluent significantly simplified the method and enhanced method reproducibility.

Keywords: Environmental Analysis, Food Safety, Ion Chromatography, Method Development
Application Code: Environmental
Methodology Code: Liquid Chromatography
Haloacetic acids (HAAs) and trihalomethanes (THMs) are two groups of commonly found water disinfection by products (DBPs). Iodinated DBPs are much more toxic than their chlorinated and brominated analogs. Peracetic acid (PAA) is a strong antimicrobial disinfectant that has the potential to reduce THMs and HAAs formation. In this study, the formations of HAAs and THMs, especially the iodinated forms, have been investigated during PAA disinfection. The formation under different iodide concentrations, pHs, and contact times were systematically studied. Two types of PAAs containing different compositions of PAA and H2O2 were used, and chlorine disinfection was also tested in parallel as comparison. THMs were detected by a newly optimized SPME-GC/MS method. HAAs were analyzed by a recently developed HPIC-MS/MS method. Results show that the ratio of PAA and H2O2 concentration significantly affected the formation of THMs and HAAs. During PAA disinfection with lower PAA than H2O2, no detectable levels of THMs and HAAs was observed. During PAA disinfection with higher PAA than H2O2, low levels of monoiodoacetic acid, diiodoacetic acid and iodoform were formed, and enhanced with the increase of iodide concentration (up to 240 µg/L).

This study was supported by Missouri Department of Natural Resources.

Keywords: Environmental Analysis, Environmental/Water
Application Code: Environmental
Methodology Code: Liquid Chromatography/Mass Spectrometry
UV filters are increasingly used in personal care products (PCPs) due to concerns about health effects associated with exposure to UV solar radiation. Organic UV filters can be found in sunscreens and other PCPs (e.g. deodorants, cosmetics, lipsticks). In swimming pools, swimmers release various organic inputs, including PCPs, that can react with chlorine leading to the formation of disinfection byproducts (DBPs), which have been linked to adverse health effects. Little is known about the potential of UV filters to act as precursors for the formation of DBPs in chlorinated swimming pools filled with seawater. In seawater pools, due to high bromide content, chlorine is transformed into bromine. The latter, a more potent oxidant compared to chlorine, reacts with organic compounds leading to formation of brominated DBPs, known to be far more toxic than their chlorinated analogues. In the present study, the reactivity of five commonly used organic UV filters (benzophenone-3, benzophenone-8, dibenzoylmethane, ethylhexyl methoxy cinnamate and octocrylene) in chlorinated seawater was investigated by conducting laboratory-controlled chlorination experiments. Four UV filters were found to react with chlorine in seawater leading to the formation of bromoform as a stable final byproduct. The yields of formed bromoform were highest for the benzophenone UV filters BP-3 and BP-8. Analysis of extracts of chlorination experiments using ultrahigh performance liquid chromatography coupled to quadrupole time-of-flight mass spectrometry (UPLC/Q-ToF-MS) allowed the separation and identification of several brominated byproducts. MSMS analysis were conducted to perform structural elucidation of the detected byproducts. Based on the identified byproducts, transformation pathways of the reactive UV filters were proposed.

Keywords: Environmental Analysis, Environmental/Water, Liquid Chromatography/Mass Spectroscopy, Mass Sp
Application Code: Environmental
Methodology Code: Liquid Chromatography/Mass Spectrometry
In the area of ion chromatography of cations, methanesulfonic acid eluent generated using the Thermo Scientific\textsuperscript{TM} Dionex\textsuperscript{TM} electrolytic eluent generator and suppressed conductivity detection provide very high detection sensitivity as well as the lowest noise, resulting in substantially lower detection limits. Over the years, the evolution of stationary phases designed for use with methanesulfonic acid has proceeded in a number of different directions. Various types of cation columns use combination of organic acids and DVB based resin with a wide range of surface areas to achieve separations suited to various applications. Recently a new cation exchange phase consisting of a novel tri-monomer system containing three different acid groups has been developed for use in suppressed ion chromatography with methanesulfonic acid eluent. This new hybrid phase utilizes multiple types of cation-exchange sites homogeneously distributed throughout the stationary phase. The choice of monomer, co-monomers and resin properties such as size, surface area and pore size makes it possible to achieve the necessary selectivity and separation.

In this presentation, we will discuss various cation column chemistries as well as separation and selectivity for various sample types. We will also demonstrate recent developments in resin technology which have allowed the use of 4 μm resin particles for cation exchange columns. The benefits of columns packed with smaller particles include higher chromatographic efficiency, better resolution or faster analysis time, easier integration and more reliable analytical results.

Keywords: Environmental, Environmental/Water, Ion Chromatography, Ion Exchange
Application Code: Environmental
Methodology Code: Liquid Chromatography
The use of surrogate compounds to measure method performance in Gas Chromatography/Mass Spectroscopy (GC/MS) methods for environmental monitoring is not a new practice. All EPA-approved methods require the use of three to six compounds; however only a few are deuterated analogs of target analytes. Deuterated analogs are more representative of target analytes, thereby providing more information regarding matrix effects while measuring the accuracy and precision. Since 2001, the EPA Office of Superfund Remediation and Technology Innovation’s Contract Laboratory Program (CLP) has required laboratories to add over a dozen deuterated monitoring compounds (DMCs) to each sample, all analogs of target analytes. Developed to improve data quality used in decision-making processes, this approach ultimately reduced the cost to the Superfund Program. This presentation will show, using over 160,000 data points from a recent study, how incorporating more DMCs into EPA-approved GC/MS methods has improved data quality and provided cost savings to the Agency, and how it may benefit the entire analytical chemistry community.

Keywords: Environmental Analysis, Gas Chromatography/Mass Spectrometry, Organic Mass Spectrometry, Qual
Application Code: Environmental
Methodology Code: Chemical Methods
# Food Identification (Half Session)

**Determination of Carbohydrates and Organic Acids in Kombucha by Ion Chromatography**

Fermentation of sugared tea with a symbiotic culture of acetic acid bacteria and yeast (tea fungus) yields kombucha tea which is consumed worldwide for its refreshing and reported beneficial properties to human health.

Sucrose is the most common carbon source in kombucha fermentation. During the fermentation process, sucrose is converted, biochemically, into fructose and glucose, and these are metabolized to gluconic acid and acetic acid, which are present in the drink. Though most of the sugar is converted into other components during the fermentation process, some still remains in the finished tea. Meanwhile, it is beneficial to monitor the kombucha’s organic acid profile because organic acids also affect the flavor and taste of the drink and play an important role in revealing the beneficial effects of kombucha.

High-performance anion-exchange chromatography with pulsed amperometric detection (HPAE-PAD) is a carbohydrate analysis technique that allows the determination of carbohydrates by direct detection, i.e. no sample derivatization is required. Ion chromatography (IC) with suppressed conductivity detection is the technique of choice to separate a large variety of organic acids with inorganic anions and detect them with high sensitivity while minimizing sugar and polyphenol interferences. In our work, we present HPAE-PAD analysis of kombucha samples for their carbohydrates, and optimized separation and quantification of the kombucha’s organic acids and inorganic anions. Both assays are conducted on a high-pressure IC system. These methods allow the accurate direct determination of the sugar and organic acid composition of kombucha.

**Keywords:** Carbohydrates, Food Identification, Ion Chromatography, Method Development

**Abstract Text**

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Globally, thousands of bottled water brands are available to consumers. The wide popularity of the many brands is associated with their affordability, availability, social status, and perception of quality when compared to tap water. However, the diversity of the water sources and their related geologies, and the possible influence of natural and anthropogenic pollution may contribute to differences in their chemical composition. For purposes of provenance, health and safety issues, it is important to evaluate their chemical content. A sub-regional study of the chemical composition of bottled waters available in three Midwestern states (Indiana, Illinois, and Missouri) of the USA was conducted. Samples (108) were purchased from popular retail stores along the I-70 interstate corridor to establish the variability due to their aquifer lithologies. Twenty-five brands were analyzed and the water types included artesian-Still, artesian-Sparkling, natural spring water, purified, purified and enriched, spring natural mineral water, and spring water. A dataset of over 40 parameters were acquired by ICP-MS, ICP-OES, and ion chromatographic and potentiometric titration methods. The results showed considerable variation in the hydrochemical characteristics of the waters and their compliance to USEPA / EU drinking water standards was evaluated. Exposure to trace metals through consumption of bottled water was calculated and compared to the IOM reference daily intakes and the WHO permissible weekly intakes. Furthermore, hierarchical cluster and principal component analyses were used to uncover the similarities / dissimilarities among the brands, and to understand the underlying variables that controlled the inorganic chemistry of the waters.

Keywords: Contamination, Elemental Analysis, Environmental Analysis, Water

Application Code: Food Identification

Methodology Code: Atomic Spectroscopy/Elemental Analysis
### Abstract Text

Olive oil is a high-value food oil, known to have various health benefits. However, this value makes adulteration and counterfeiting of olive oils a very attractive and lucrative venture for fraudsters. Thus there is a need to objectively authenticate the quality, purity, and region of origin of olive oils going to market. In the United States, it has been shown that the majority of samples of extra-virgin olive oil (EVOO) from top-selling national brands fail the purity tests for EVOO.

Various analytical methods such as GC and HPLC are used to monitor the chemical compositions of olive oils in order to detect anomalies. This is complicated by variations in soil and climatic conditions which alter the chemical compositions. Hence several compounds have to be detected and/or quantified with multivariate techniques.

Lately, the focus has shifted towards chemical fingerprinting using rapid spectrometric methods such as high-resolution NMR. These high-resolution NMR are costly to acquire and operate, in addition to having very large laboratory footprints. Recently, new LFBT-NMR spectrometers have been introduced. Even though these devices even do not have the resolving power of their high-field counterparts, spectra acquired could have enough information to enable rabid screening and classification of olive oils.

In this study, we apply chemometric tools to spectra obtained from LFBT-NMR with the aim of distinguishing olive oils from non-olive oils. We further investigated the potential of estimating the level of adulteration of olive oils by other food oils.

**Keywords:** Chemometrics, Food Identification, Magnetic Resonance, NMR

**Application Code:** Food Identification

**Methodology Code:** Magnetic Resonance
Testing of Fatty Acid Methyl Esters (FAMEs) not only allows for authentication of oil products, but also serves as an indicator of any adulteration. Complex FAMEs including cis/trans fatty acids, however, have historically been challenging to separate in a reasonable period of time. Commercially available cyano phase GC columns traditionally have had analysis times of nearly an hour and utilized column lengths up to 100 meters, which presents a costly, time-consuming analysis process.

In this study, we present a unique cyano-based GC stationary phase, Zebron ZB-FAME, which is optimized to improve performance for complex FAMEs analysis. Samples of commercially available canola oil, coconut oil, olive oil, and walnut oil were derivatized using a BF3-methanol reagent to convert the fatty acids to FAMEs. These derivatized oils were then analyzed on several on commercially available cyano phases, and evaluated for time- and cost-efficiency. The unique selectivity of the ZB-FAME column presented in this work provided shorter run times using a cost-effective 30 meter length, while also providing complete resolution of a 37-component FAMEs mixture. In addition to these optimizations, we discuss the possibilities of post-run bake out and column lifetime improvement offered by the column’s high upper temperature limit.

Keywords: Food Identification, Food Science, GC, GC Columns
Application Code: Food Identification
Methodology Code: Gas Chromatography
Food packaging (FP) is vital in protecting foods, improving hygiene and extending the shelf-life of packaged foods. However, during storage and handling, some chemicals from FP materials migrate into foodstuffs, presenting a source of uncharacterized chemical contamination.

The goal of this study was to identify and characterize FP contaminants migrating from the most widely used FP materials – stretch plastic films into food simulants: US FDA approved food simulant based on ethanol/water mixture and organic solvents with different polarities: ethyl acetate and hexane (to mimic lipophilic properties of fat-containing food) and acetonitrile (to extract both polar- and relatively non-polar chemicals from the FP materials).

To identify FP migrants, non-targeted analysis using a comprehensive two-dimensional gas chromatography coupled to time-of-flight mass spectrometry (GC×GC/TOF-MS) was used.

93 migrating chemicals were identified in ethyl acetate, 76 in acetonitrile and hexane, and 66 in ethanol-based food simulant. Other chemicals may also have migrated, but did not pass the NIST MS similarity threshold (>80% match similarity to the standard NIST MS library). Several classes of chemicals were identified among the migrating compounds: alkylated naphthalenes used as lubricants; polycyclic aromatic hydrocarbons (PAHs) including those on the US EPA priority pollutant list; plasticizers, polymer additives, UV filters, surfactants, adhesives, products of thermal degradation, and low chlorinated polychlorinated biphenyls (PCBs). The identified compound were characterized based on use/source, chemical properties and potential toxicity. Preliminary experiments exposing ground beef to plastic films showed potential migration patterns, and subsequent experiments are underway to identify FP migrants from plastic films into ground beef.

Keywords: Food Contaminants, Gas Chromatography/Mass Spectrometry, Identification, Mass Spectrometry

Application Code: Food Safety

Methodology Code: Gas Chromatography/Mass Spectrometry
Three is an increasing interest in the development of food packaging materials that provide active protection, intelligent communication while exhibiting biodegradability. We hereby report the molecular design, preparation and characterization of a new class of smart, biodegradable, ternary poly (amic) acid copolymers or BioTerc PAA. The polymer uses a combination of PAA, homolytic linkers (e.g. glutaraldehyde,) and small biomolecules (i.e. D-glucosamine, amino acids such as L-alanine, L-Cysteine and other ligands [e.g. –p-aminobenzoic acids]). The resulting PAA copolymers exhibit the following properties: advanced mechanical properties in the range 2-4 GPa modulus elasticity that are comparable to strong plastics (2.4 to 5 GPa), stability in common solvents, high optical transparency, impermeability to gas exchange, oil and water vapor transfer. Application of BioTerc PAA as smart food packaging materials was demonstrated for cheese, pepperoni, apple and walnut for up to 15 months. The synthesized PAA co-polymers provide superior properties which can be a candidate to meet the requirements of society’s expectations for a safer environment and sustainable future.
Food is often contaminated with non manmade toxic compounds. Some of the toxic compounds, mycotoxins, are synthesized by molds. Mycotoxins are compounds with nearly no vapor pressure and cannot be measured directly in the vapor phase. Indirect detection of mycotoxins is possible by detecting VOCs from the metabolism of the molds.

During on-site process control a complex matrix of chemical compounds of the grain silo headspace has to be expected. For pre-separation, coupling of the ion mobility spectrometer (IMS) with a gas chromatograph (GC) is necessary. Since the generated GC peaks are short in time a new type of gate-less double tube IMS is used as the detector, which can detected both positive and negative product ions simultaneously.

The focus of the work lies in identification of requirements for operation and control of GC e.g. the length of the capillary and the temperature profile. Suitable interfaces between GC and IMS have been identified. The GC is integrated directly into the drift gas loop of the IMS. Thus no additional carrier gas supply is needed and the field capability can be ensured. Requirements for the appropriate operation of the GC-IMS were identified.

Grain with and without contamination with different molds were investigated by measuring the VOCs by laboratory analysis with GC MS. Compounds that correlated with the molds/mycotoxins were identified and pointed out as target substances for the on-site process GC-IMS system. We will present measurements that demonstrate the ability of the GC-IMS system to separate and detect this target substances.

Keywords: Food Safety, Gas Chromatography, Instrumentation
Application Code: Food Safety
Methodology Code: Gas Chromatography
A laboratory informatics solution (e.g. LIMS, ELN, CDS) is only as strong as the weakest part of its foundation. The foundation for the implementation of a laboratory informatics solution is the user requirements document that is generated to identify exactly what the system is intended to do. The requirements document is used extensively throughout the selection, development, and implementation of the solution and in the end, is the yardstick used to measure its success. Unfortunately, many times this document is not created with the thoroughness and attention to detail that its importance deserves. Too often it is looked at as a checkbox exercise where many organizations take the approach that they will have plenty of time to correct any inconsistencies as the project evolves. Unfortunately, it is often the case that many decisions have already been made based on the document contents before the realization occurs that the requirements documented were not detailed enough or accurately reflect the actual business needs. The result of such neglect can be time and cost overruns, a system that is inefficient, users who are unhappy, and even the possibility of total project failure.

This presentation will take a look at proven, thorough, yet sensible approaches to ensuring that your business is in the best position to create solid user requirements for your informatics system. Overcoming different obstacles such as lack of resources, inconsistent work processes, lack of informatics “experts”, and lack of business motivation within the company will also be addressed. In addition to generating a solid, well thought out user requirements document, the presentation will provide ways to also leverage the requirements generation exercise to improve communication, morale, and work processes, all while having a little bit of fun.

Keywords: Laboratory Informatics, Quality, Sample & Data Management
Application Code: Laboratory Management
Methodology Code: Laboratory Informatics
Your organization has determined that it needs a Laboratory Information Management System (LIMS) in order to attain and support its laboratory and business goals. Your capital appropriations cycle is rapidly approaching and your management is requiring you to submit a comprehensive budget for this project. There will be no going back to the trough! Will you submit the right amount in order to be successful? What factors, considerations, and costs should you be exploring beyond those of LIMS licensing, hardware, and standard implementation?

This talk will examine the different types of LIMS licensing schemas available today and how these options will impact your budgeting. Additionally, we will explore other factors that need to be considered when budgeting for a successful LIMS project. Lastly, we will explain how informatics strategy and planning can be utilized to ensure that the complete LIMS project cost picture is developed and detailed.

Keywords: Laboratory Automation, Laboratory Informatics, LIMS, Software
Application Code: Laboratory Management
Methodology Code: Laboratory Informatics
Low cost microcontroller (MCU) boards such as Arduino and Raspberry Pi have revolutionized the field of open source analytical instruments; and now with published reports of instruments such as potentiometers [1], Raman spectrometers [2] etc; there is a pressing need of a data acquisition and analysis software for such MCU based instruments.

This talk introduces one such graphical user interface program called ChemAnalysis workbench, based on an Eclipse rich client platform (RCP), and written in java programming language, it is highly modular with an extensible design. The Eclipse environment has proven to be a very robust system, and is extensively used by organizations such as IBM, SAP, NASA as a framework for their software applications. Eclipse building units are called plug-ins, and each functionality is developed and deployed as a separate plugin, which allows an instrument designer to quickly adapt ChemAnalysis for a custom application in case its not supported by default.

ChemAnalysis has a data acquisition layer, where it reads data from an instrument's electronics through USB port; a data visualization layer, which plots the data into 1D, 2D and 3D; and a data analysis layer which provides scripting support using python and R for advanced data analysis. ChemAnalysis also comes with default scripts to run the commonly used data analysis and statistical functions. All of this is packaged as a graphical user interface for a user friendly experience.

Companies are correct to follow a risk based approach to protecting their SDMS data by backing it up at regular intervals. However, too often companies take a more conservative, risk adverse approach and back up more standalone data and meta-data than necessary. When an SDMS sweep covers more than just instrument raw data and metadata, not only will the amount of required server disk space become quite extreme, data searches and restorations become much more time consuming and cumbersome.

A better solution is implementing a SDMS platform that is lean, well thought out, and easily searchable and restorable. This talk will cover ways to achieve that state and the importance of SDMS setup and configuration. We will cover topics such as logical search filters and prudent, client specific meta tags, as well as, wise SDMS scan line selections. The objective of this talk is to provide Laboratory Management & Supervisors and SDMS Administrators & End Users with the best SDMS back up practices.

Keywords: Informatics, Lab Management, Laboratory, Scientific Data Management
Application Code: Laboratory Management
Methodology Code: Laboratory Informatics
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Abstract Text

Bad software that is well implemented can be more beneficial to the laboratory end user than good software that is poorly implemented. It is easy to get swept up in the bells and whistles of new laboratory software like LIMS, ELN, or LES and assume that the software will be a magic bullet for laboratory process or data management pain points. Unfortunately, there is no magic bullet, but there are ways to avoid the pitfalls of such an assumption. Modern software can automate and enforce almost any process, but there is no value in automating poor processes.

This presentation will tap decades of CSols’ cumulative laboratory experience and explain high level steps that laboratories and IT organizations can take for more successful—and therefore higher value—informatics implementations. Often-overlooked tasks such as static data organization, process optimization, terminology standardization, and planning for post-implementation validation will be addressed. Other important pre-implementation planning Dos and Don’ts will also be covered.

Keywords: Laboratory Informatics, LIMS, Quality, Sample & Data Management
Application Code: Laboratory Management
Methodology Code: Laboratory Informatics
Successfully implementing your LIMS and attaining high levels of acceptance and utilization by the stakeholders is a daunting and difficult task. There are so many aspects, factors, needs, and desires that need to be optimized, prioritized and managed that it is easy to “fail to see the forest for the trees”. Instituting a well thought out, proper LIMS Project Governance Model which clearly defines the purpose, benefits and detailed roles and responsibilities of your LIMS team is essential. Additionally, it is imperative to establish and religiously follow a Communication Policy and Plan to ensure that all your LIMS stakeholders be kept informed and on-board during your LIMS implementation and beyond.

This talk will elucidate a best practices derived LIMS Project Governance Model and Communication Plan and Policy. Further, due to the complex nature of LIMS teams which today are routinely made up of multi-sourced personnel (i.e. internal resources, LIMS vendor resources, and 3rd Party resources) the need and benefit of parallel roles will be explored and insights as to how to manage multi-sourced teams will be shared.

Keywords: Laboratory Automation, Laboratory Informatics, LIMS, Software
Application Code: Laboratory Management
Methodology Code: Laboratory Informatics
Verifying the accuracy of data in a study is always challenging, especially when reporting data for studies with a large number of samples being analyzed using a high-throughput multi-analyte method. The whole blood volatile organic compound (VOC) method used by the CDC in support of the National Health and Nutrition Examination Survey (NHANES) involves analysis of over 3,500 blood samples per cycle for up to 44 VOCs, generating over 150,000 results per report. This data is analyzed using chromatographic integration software executed by an analyst, who ensures correct calibration and that peaks have been properly integrated. To account for any errors made during analysis, a custom Microsoft Access reporting system (DataPro) was built to flag 25 different errors that can be identified based on retention time, ion ratios, curve fit characteristics, and other important data parameters. DataPro compiles appropriately labeled tables with the errors it identifies, prompting the analyst to resolve the errors or mark the sample for repeat. Samples marked for repeat analysis are passed to another reporting system (RepeatPro) that queues the samples. RepeatPro allows the user to queue and analyze single analyte-sample pairs, then feeds repeated results back to DataPro for the comparison of initial and repeated results. Once data analysis has been completed, run data is uploaded to a web-based database capable of accepting and verifying error reports generated by DataPro.

Keywords: Laboratory Informatics, Scientific Data Management, Software
Application Code: High-Throughput Chemical Analysis
Methodology Code: Laboratory Informatics
You’ve heard that SAP QM now has full functioned LIMS-like capabilities and that a separate LIMS application is no longer required. You also know that within some industries it is typical for both SAP QM and LIMS to be implemented. So is SAP the only system you need to operate both your enterprise and your QC Lab?

Since the introduction of SAP QM its power has increased and now reaches into the sample management, stability studies and instrument calibration domains. During the same time period LIMS has strengthened its capabilities and new specialized systems such as SDMS, ELN and LES have appeared in the Lab Informatics landscape. The battle for dominion has entered a new phase.

This presentation will examine the evolution of SAP and SAP QM and describe how SAP QM fits into to the overall Lab Informatics landscape. In a factual, but light hearted manner, we will compare systems to Rings of Power and SAP to the One Ring as described by Tolkien in the Lord of the Rings:

One Ring to rule them all,
One Ring to find them,
One Ring to bring them all
And in the darkness bind them.

Each descriptive line will be examined in turn and whether and how it may apply to SAP and your laboratory will be described. The presentation will conclude with an examination of darkness and light and demonstrate that, depending on your viewpoint, they may very well be the same.

Keywords: Informatics, Laboratory Automation, Laboratory Informatics, LIMS
Application Code: General Interest
Methodology Code: Laboratory Informatics
The recent application of urinary metabolomics to cancer biomarker discovery has revealed a number of putative biomarkers and aberrant metabolic pathways. Among these, pteridines, folates, and modified nucleosides have appeared to be the most promising for eventual clinical implementation in noninvasive risk screening and early detection assays. However, the availability of analytical methods suitable for their simultaneous determination is lacking, which has precluded biomarker validation in targeted population-based studies. We have therefore developed an ultra-fast liquid chromatography – tandem mass spectrometry (UFLC-MS/MS) method for the simultaneous determination of 28 biologically relevant pteridines, folates, and modified nucleosides. The resulting method features rapid analysis times (6 min), minimal sample preparation, the ability to characterize the metabolites in their native oxidation states without chemical modification, and the separation of seven pairs of structural isomers. The validated method possessed excellent sensitivity (method detection limits: 0.01 µg/L – 0.5 µg/L), good reproducibility (RSD: 2-9%), and good accuracy (85-109%) based on spiked recovery studies. Finally, 150 clinical urine specimens collected from age-matched breast cancer case-controls were profiled with the newly developed method. In this study, we demonstrated that the newly developed technique can be used to support biomarker validation of these important metabolites in future clinical studies. The detailed findings of the clinical study, including population ranges and the separation method, will be presented at the conference.

This study was supported by a National Science Foundation Graduate Research Fellowship.
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. Current methods for the determination of urinary amino acids often require complex chemical derivatizations or are only applicable for individual amino acids. The lack of available methods for this purpose prompted us to develop a high-performance liquid chromatography – tandem mass spectrometry (HPLC-MS/MS) method for the simultaneous determination of seven amino acids, including alanine, asparagine, leucine, isoleucine, valine, proline, and cysteine along with two oxidized amino acid derivatives, cystine and 4-hydroxyproline. The resulting method was 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 newly developed 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 may 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 cancer detection. The detailed method development and results of this study, such as method detection limit and quantitation limit, and 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: Liquid Chromatography/Mass Spectroscopy, Metabolomics, Metabonomics, Method Development, Bioanalytical

Methodology Code: Liquid Chromatography/Mass Spectrometry
Quantitative and effective techniques for blood sampling and analysis are required to meet the needs of the medical and bioanalytical communities. Traditional blood draws are inherently used because of the amount of blood typically desired to be withdrawn from the patient has been standardized by the venipuncture blood draw. However, in practice some tests only use a portion of blood, with the remainder being discarded. Dried Matrix Spots (DMS) greatly reduce the volume of collected blood, thus enabling more frequent and easier procurement with a finger stick instead of a venous blood draw. Additionally, DMS cards can be legally transferred across international borders. This sampling technique may enable physicians to monitor the progression of a patient’s treatment by providing a means for the patient to do in home sampling and then send the spotted cards to analytical laboratory for analysis. Traditional DMS workflows require tedious sample preparation and is therefore more time consuming than the methodology used in this study. This study presents a unique approach to characterizing DMS by using on card “clamp-and-elute” technology with quantitation using isotope dilution mass spectrometry. By utilizing technology to equilibrate the sample with the isotopically labeled analogue quantitation is enabled in DMS. This technique is applied to analytes that are medically relevant allowing diagnostic and patient monitoring for many areas of healthcare including wellness monitoring.
Peptide mapping is the chromatographic separation of the fragments derived from controlled digestion of a protein. It is used to identify a protein and to measure the proportion of modified amino acid side chains within a protein sample. Comprehensive mapping is difficult since the mixture contains many components representing a wide range of properties. Reversed-phase chromatography has been the most successful approach to peptide mapping. This separation requires removing charges from the peptides. Separations developed near the pK’s of the amino acid side chains can provide an additional source of selectivity. Small changes in pH can change the chromatographic selectivity since side chain pK’s can vary by a half of pH unit. The small incremental adjustments of the mobile phase pH can, therefore, be effective. This manipulation has not been employed in peptide mapping because the preparation of buffered mobile phases is time consuming, labor-intensive and variable. We have developed techniques and software to facilitate the preparation of buffered mobile phases from concentrated stocks. Mobile phase buffers are prepared on-demand using the proportioning capabilities of the four solvent delivery technique of the system. To provide peak tracking and identification, the buffers are volatile and transparent. Electrospray MS detection can, therefore, be combined with UV spectral detection using a photodiode array detector. This mobile phase manipulation has been applied to digests of several different proteins including monoclonal antibodies. Useful changes in chromatographic selectivity have been observed with changes of a few tenths of a pH unit. The automated preparation of mobile phases with different pH properties helps to ensure the optimum selectivity for peptide mapping.

Keywords: Analysis, Liquid Chromatography/Mass Spectroscopy, Peptides, Protein
Application Code: Bioanalytical
Methodology Code: Liquid Chromatography/Mass Spectrometry
Abstract Text

Folate-derived pteridines are putative biomarkers for early cancer detection and risk assessment. However, the clinical applicability of these compounds remains highly controversial, despite strong clinical evidence demonstrating their association with numerous malignancies, because of the unknown pathophysiological function of pteridine derivatives in cancer development and progression. Current understanding on the biochemistry and molecular pathology of pteridines has been limited by the availability of quantitative analytical methods suitable for their comprehensive characterization in cellular matrices. We therefore developed a high-performance liquid chromatography – tandem mass spectrometry (HPLC-MS/MS) method for the simultaneous determination of 24 intracellular pteridine derivatives, their anticipated isotopic-labelled patterns ([M+2][\sup]...[M+6][\sup]), and two stable isotope tracers ([\sup]15[\sub]N[\sub]5]-folic acid and [\sup]15[\sub]N[\sub]5]-guanine) from cell lysates. This work extended our previous efforts to quantify intracellular pteridines by switching from semi-quantitative analysis to quantitative analysis, optimizing the extraction of intracellular lysates, removing oxidative pretreatments, and reducing the minimum cell count required for analysis. The newly developed method was validated through evaluation of spiked recoveries (78-107%), reproducibility (2-16% RSD), method detection limits (0.05 µg/L – 1 µg/L), and finally application to A549 non-small cell lung cancer cells. The validated method was used to support metabolic flux analysis of the folate-derived pteridine biosynthetic pathway to comprehensively map, for the first time, the interconnectivity of pteridine derivatives. The detailed methods and results will be presented at the conference.

This study was supported by a National Science Foundation Graduate Research Fellowship.
Forced degradation studies are typically performed using liquid chromatography (LC) and ultraviolet (UV) detectors to understand the degradation pathway of pharmaceuticals and that the method is able to demonstrate specificity. Given the range of impurities and their chemical and physical properties, mass balance studies can be challenging. These challenges may be due to co-elution of impurities and the active pharmaceutical ingredient (API), the presence of undetected components, or differences in response of the impurities to the API: all of which can lead to poor recoveries.\textsuperscript{1} To address these difficulties, multiple orthogonal detectors based on different principles can be used to measure or identify compounds with different chemical or physical properties.

In this presentation we will evaluate mass balance using a triple detection system consisting of a photodiode array (PDA), evaporative light scattering detector (ELSD) and a mass detector (MS). These studies will be performed in two parts. First, the relative response factors of impurities will be evaluated. Relative response, which is based on the ratio of UV peak area to concentration (mass), will be calculated using the slopes of the calibration curves. This well-established technique will then be compared to a method using the ratio of the response in UV to ELSD detection. In the second part, the relative response factors will then be used to perform mass balance. Specifically, mass balance recoveries of the degraded samples will be determined using chromatographic software tools. The degradation pathway will then be confirmed through the identification of impurities and their by-products using a mass detector.


Keywords: HPLC, Liquid Chromatography, Liquid Chromatography/Mass Spectroscopy, Pharmaceutical
Application Code: Pharmaceutical
Methodology Code: Liquid Chromatography/Mass Spectrometry
Genotoxic impurities (GTIs) have potential to react with DNA and induce genetic mutation, which may consequently lead to cancer. Accurate identification and control of these toxic impurities during the drug development process is an important aspect to ensure product quality and minimum risk to the patient safety. Regulatory guidance on genotoxic impurities requires that the analytical procedures must have "as low as reasonably practicable" (ALARP) detection limits. This will enable assessment of process capability and tracking the fate and purging levels of the impurities during the drug development process.

Imatinib mesylate is an anti-neoplastic (anti-cancer) drug used for the treatment of chronic myeloid leukemia and gastrointestinal stromal tumor. Two process impurities, 4-[(4-Methyl-1-piperazinyl) methyl]benzoic acid dihydrochloride and N-(5-Amino-2-methyl phenyl)-4-(3-pyridyl)-2-pyrimidineamine, are classified as genotoxic impurities based on the structural alerts and must be monitored during the synthesis process.

In this work, we describe a sensitive and rapid UPLC method coupled with mass detection for the quantitative determination of two genotoxic impurities of the imatinib mesylate drug substance. We will investigate and compare sensitivity achievable with two mass detectors, a single quadrupole (ACQUITY QDa) and a tandem quadrupole (Xevo TQ-S micro). In addition, we will evaluate method linearity and specificity with each mass detector. We will show that the tandem quadrupole detector is ideal for ultra-sensitive residual detection and quality monitoring of genotoxic impurities in the drug substances, while the QDa provides a robust and sensitive platform suited for routine monitoring of drug substances in late stage development or QC environments.

Keywords: Liquid Chromatography/Mass Spectroscopy, Quantitative, Tandem Mass Spec
Application Code: Pharmaceutical
Methodology Code: Liquid Chromatography/Mass Spectrometry
There are many steps during the manufacturing process of an active pharmaceutical ingredient (API) where impurities can be introduced, whether as reagents, byproducts, intermediates, etc. 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, which frequently do 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 analytical methods. Additionally, mass spectrometry is known to be suitable for use with both RPLC and SFC methodologies.

Ondansetron is a pharmaceutical used in the prevention of nausea and vomiting, and contains two process impurities that are potentially mutagenic, imidazole and 2-methyl imidazole. 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.
Pyrantel is a thiophene based antinematodal active pharmaceutical ingredient (API). It is used to treat and prevent the occurrence of intestinal parasites in dogs in a new oral endectoparasitiside chewable product. A stability-indicating HPLC method was developed for the assay of pyrantel and estimation of its degradation compounds in a new oral endectoparasitiside chewable product. Ground sample was extracted with the extraction solvent composed of 10% Water (H2O), 86% Methanol (MeOH), 2.8% Triethylamine (TEA) and 1.2% Acetic acid (AcOH) (v/v/v/v). Chromatographic separation of Pyrantel and its degradation compounds was achieved by using an isocratic elution at a flow rate of 1.5 mL/minute using Altantis Hilic Silica (50 mm x 4.6 mm i.d., 3 µm particle size) as the primary column and Halo Hilic (50 mm x 4.6 mm i.d., 2.7 µm particle size) as the equivalent column at 40°C. The mobile phase consists of 94.5% Acetonitrile (ACN), 2%water (H2O), 1% Triethylamine (TEA) and 2.5% Acetic acid (AcOH) (v/v/v/v). A UV detector at 288 nm was used to detect the analytes. The total run time for this method is 6 minutes. The new method was successfully validated 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 Pyrantel related compounds (from pyrantel and from each other) that are present in stressed samples under heat, light, base, acid and oxidation. This fast HPLC method is ideal for QC labs to conduct routine test for the assay of Pyrantel and estimation of its degradation compounds in the oral endectoparasitiside chewable product.

Keywords:  HPLC, Pharmaceutical, Validation
Application Code:  Pharmaceutical
Methodology Code:  Liquid Chromatography
Method translation software is very useful for GC applications when you need to change carrier gas type, column dimensions, phase ratio (film thickness), column outlet pressure, or carrier gas flow rate. The main assumptions are that you are not changing stationary phase chemistry and that the elution order of your current method is satisfactory.

Method translation software allows you to modify or enhance a current GC method while ensuring that analyte retention order is maintained. For instance, if you want to speed up an analysis or change detectors (such as from an FID to an MSD) you can use the method translator to yield a perfectly scaled version of the original.

Traditional GC methods will be translated for use on a new GC platform and the resulting chromatograms will be compared to the originals. Tips and guidance on how to achieve optimal results using the new platform will also be discussed.
Ginger is an indispensable ingredient in cooking and home remedies. With the confluence of popularity of ginger as a dietary supplement and the global sourcing of the commodity, attention is drawn to its safety, and especially to its heavy metal contamination. Arsenic is a regulated toxic substance that is regularly tested in agricultural products. However, not all forms of arsenic are toxic. Current regulations focus on the most toxic species of inorganic arsenics: arsenous acid (AsIII) and arsenic acid (AsV). In support of dietary safety measurements of ginger, NIST is developing a standard reference material (SRM) 3398 Ginger Rhizome. However, there is no reported procedure on assessment of toxic arsenic in ginger.

A procedure was developed to reproducibly extract the toxic arsenic species from ginger and then to identify and quantify the arsenic species. The extraction procedure was optimized for solvent, temperature, and equilibration time. Arsenic species in the extract were identified based on the retention time of the species, and the quantities were determined by liquid chromatography-inductively coupled plasma mass spectrometry (LC-ICP-MS). Separately, arsenic in the ginger and the ginger extract was determined by ICP-MS for evaluation of extraction efficiency and for validation of the speciation measurement. SRM 3398 was found to contain approximately 50 mg/kg arsenic, of which 4 mg/kg was extracted at room temperature and determined to be inorganic arsenic. Two brands of ginger powder and one fresh ginger from local grocer were found to contain arsenic that were orders of magnitude lower than that in SRM 3398, suggesting that SRM 3398 may not be representative of ginger found in the US market. The amount of extractable arsenic increased exponentially with the temperature, and the implications of this effect will be discussed.

Keywords: Food Contaminants, Food Safety, ICP-MS, Speciation
Application Code: Food Contaminants
Methodology Code: Liquid Chromatography/Mass Spectrometry
Ensuring drug product quality and patient safety is the primary concern of pharmaceutical industry and regulatory agencies around the world. Significant effort goes into delivering safe, efficacious medications of desired quality. A battery of analytical tests are performed on the active pharmaceutical ingredients (API) and Drug product among which the assay and related substance method is the most critical test.

The pharmaceutical industry relies on age-old technologies like DAD and or MS detection to ensure the purity of the API. Although, this might be adequate, the approach has shortcomings as chemical components eluting in and around the main component (API) are usually structurally similar and or isomers of main component and might be inadequate to detect residual, co-eluting impurities. The limitations become more blatant as we move on to modern day medications like monoclonal antibody drug conjugates (ADC’s) used in oncology. Complexity in the synthesis, chemical reactivity and instability of the linker drug pushes the limits of modern day chromatography mandating the use of multi-dimensional separation. A detailed characterization of the linker drug for potential impurities upstream is critical to limit, minimize its impact in downstream conjugation.

The presentation will cover the applications of two-dimensional liquid chromatography in the qualitative and quantitative analysis of residual impurities co-eluting with the new linker drug, assess lot-to-lot variability and reproducibility of two-dimensional liquid chromatography. Additionally, 2D-LC method development strategy for these complex samples will be presented.

Keywords: Chromatography, Liquid Chromatography/Mass Spectroscopy, Pharmaceutical

Application Code: Pharmaceutical

Methodology Code: Liquid Chromatography/Mass Spectrometry
Monoclonal antibodies are among the most successful and most expensive therapeutic agents. Rapid, inexpensive antibody capture should decrease the cost of both production and detection of these remarkable drugs, and affinity membranes can potentially protein more rapidly than traditional bead-based methods without the large pressure drops of columns. Furthermore, membranes modified with polyelectrolyte films exhibit higher capacities than traditional membranes for protein capture. In this research, we covalently linked antibody-binding peptides to poly(acrylic acid) adsorbed in porous nylon membranes. Specifically, we immobilized two peptides: a mimotope (denoted K19) for the specific binding site of the therapeutic antibody Herceptin (Trastuzumab) and a Fc (fragment, crystallizable) binding peptide (denoted KK12) These peptides contain lysine termini to facilitate amide coupling to poly(acrylic acid), and the peptide loadings in poly(acrylic acid)-modified nylon membranes were 57.6 ± 0.3 mg of KK12 per mL of membrane and 27.4 ± 4.0 mg of K19 per mL of membrane. The KK12-modified membranes captured 8.4 mg of Herceptin and 10 mg of Avastin (Bevacizumab), another therapeutic antibody, per mL of membrane. Similar binding capacities for Herceptin and Avastin are consistent with an Fc-binding peptides. The antibodies eluted in 100 mM Gly (pH 2.7), but the membranes were not reusable. Inexpensive Fc-binding membranes may offer a rapid, lab-scale alternative to protein A and protein G columns for antibody analysis. K19-modified membranes capture 16 mg of Herceptin per mL of membrane and showed minimal binding of Avastin. Furthermore, these membranes selectively isolated Herceptin from human serum and showed negligible significant non-specific adsorption. Thus, current research aims to develop membrane-based antibody assays for rapid, inexpensive quantitation of therapeutic antibodies in serum.
Despite >40 yr of SPE using LC sorbents, LC principles have been ignored. At the root of this is the lack of flow control in the SPE devices in use today. Whether using vacuum or pneumatic pressure, the changing volume of liquid above the sorbent changes the flow rate through the sorbent. When SPE is performed in parallel, this effect is exacerbated because each SPE device (or well) has a different resistance to flow. The result of all the variable flow is considerable variation in results. Internal standards must be used to achieve meaningful results with all single use SPE devices and overall data must be judged based on the worst case scenario (flow far from optimal). Furthermore, achieving 100% absolute recovery against external standards, the gold standard in demonstrating the absence of matrix effects, seems to be lost.

With the invention of the ITSP SPE device, all of the above is changed. The ITSP SPE cartridge is designed around the syringe in order to achieve both automation (SPE cartridge transport) and accurate flow (performance like LC columns). The result of its use with a CTC/PAL autosampler is total automation of SPE and LC/MS/MS (or GC/MS/MS in parallel) measurement in a single parallel workflow (SPE & LC/MS/MS in parallel). SPE method development is a matter of systematically applying chromatographic principles. van Deemter curves can be measured for SPE cartridges (proving reversible adsorption/desorption equilibria) and SPE can be performed at the optimal flow rates to achieve 100% absolute recovery versus external standards. Since this SPE cartridge is a micro SPE device, sample dry down is no longer needed to achieve sample enrichment as high as 200x. Finally, SPE can be performed with single use devices efficiently, economically, and with a performance level that matches all chromatographic knowledge gained in the last 50 years. Compelling examples of clinical measurements using reverse phase and ion exchange SPE will be provided.

Keywords: Clinical Chemistry, Laboratory Automation, Liquid Chromatography, Solid Phase Extraction
Application Code: Clinical/Toxicology
Methodology Code: Sampling and Sample Preparation
Arsenic is a common groundwater contaminant, with health effects including cardiovascular disease, skin lesions, and a range of cancers. In regions like Bangladesh with heavy arsenic contamination, the suitability of a particular water source for drinking purpose depends on the level of arsenic. However, accurate quantification of arsenic requires the use of techniques such as atomic absorption spectroscopy (AAS), inductively coupled plasma-atomic emission spectroscopy (ICP-AES), or inductively coupled plasma-mass spectrometry (ICP-MS). In countries such as India, the capital cost and required expertise for these techniques limits their use to centralized laboratory facilities, with the result that routine water samples are frequently only tested using measurements available at local labs and not for contaminants such as arsenic which require elemental analysis instrumentation for accurate measurement.

In this work, we present the use of iron-oxide xerogels for adsorption, dry storage, and release of arsenic from water samples. Iron oxide sorbents’ ability to remove arsenic from water is well-documented, and epoxide-based sol-gel chemistry facilitates rapid, simple, inexpensive preparation of high-surface-area nanoporous iron oxide. Iron oxide xerogels prepared via epoxide-based sol-gel chemistry and characterized via Brunauer-Emmett-Teller (BET) and Barrett-Joyner-Halenda (BJH) analyses reliably exhibited mesoporous morphologies with surface areas of at least 330 m²/g and pore volumes of less than 0.3 cm³/g, resulting in an excellent geometry for compact adsorption and storage of aqueous arsenic. By demonstrating the ability for iron oxide xerogels to reliably adsorb arsenic from water samples, be stored as a dry sample matrix, and then release the arsenic back into solution, we enable the development of dry sampling protocols where arsenic would be collected from routine drinking water samples by nanoporous iron oxide sorbents at local labs, and the sorbents would be dried and easily shipped to centralized labs where the adsorbed arsenic would be released and quantified.

Keywords: Environmental/Water, Sample Preparation, Sampling, Water
Application Code: Environmental
Methodology Code: Sampling and Sample Preparation
Abstract Text

The complex composition of difficult matrices such as dry commodities presents a challenge when performing chromatographic analysis of pesticides. Residual pigments and oils from such matrices can contaminate both GC/MS and LC/MS systems, and also result in significant matrix effects. Therefore, an effective cleanup method is needed prior to the injection of extracts onto GC or LC systems. Standard QuEChERS methodology does not offer the cleanup capacity required for the analysis of high background samples. Recently, a novel dual-layer cartridge was designed for the cleanup of acetonitrile extracts from difficult matrices and dry commodities (spices, tea, etc.) prior to pesticide residue analysis. The top bed consists of a mixture of PSA, C18 and a novel graphitized, spherical carbon. This carbon was engineered to remove sufficient pigmentation while allowing for better recoveries of planar pesticide residues, without the need for toluene in the elution solvent. The bottom layer of the cartridge contains a zirconia-coated silica. This sorbent removes oily residues and provides additional retention of some pigments. This GC/MS/MS and LC/MS/MS analysis of a wide range of pesticides of different polarities and classes will be demonstrated in this study. Recovery, reproducibility, and background removal in the analysis of various pesticides from extracts of difficult matrices will be discussed.
A vapor delivery device was designed to safely contain the solid or liquid components of homemade explosives and deliver the component vapors for instrumental sampling and analysis. Within the device, vapors from each of the separated components mix as they are carried through the device by flowing air. The resulting mixed vapor is representative of that which would be achieved from the actual mixed explosive material. For sampling, air flows from an external source, through the device, carrying the mixed analyte vapor towards the instrument of choice. Component materials are held in up to four individual, removable vials allowing for ease in the alteration of the component vapor ratios by simply adding or removing individual vials, or by placing restricting lids on the vials to lessen the vapor output for specific components. Additionally, a thermal water jacket surrounding the entirety of the device allows for its heating or cooling from an external water circulator.

The device was evaluated first with surrogate compounds, then with two types of homemade explosives, to include a binary explosive mixture and a peroxide explosive. To evaluate the device, vapors were cryo-trapped with an online sampling system and analyzed by gas chromatography / mass spectrometry. It was determined that the device yields reproducible vapor concentrations of both single and mixed components, and the ratio of these vapors can be easily adjust to mimic varying forms of homemade explosives.

Keywords: Forensic Chemistry, Gas Chromatography, Sampling, Volatile Organic Compounds
Application Code: Homeland Security/Forensics
Methodology Code: Sampling and Sample Preparation
Sampling and Sample Preparation - MS and IC
Extraction and Analysis of Organochlorine Pesticide Residues in Fatty Matrix by Lipid Removing Sorbent and GC/MSMS

Most persistent organic pollutants (POPs) are organochlorine pesticides (OCPs) and have been banned in many countries like North America, Europe and many countries in South America, in accordance with Stockholm Convention in 1980s. Contamination routes can lead to bioaccumulation of persistent pesticides in food products of animal origin such as meats, fish, eggs, milk and processed food that incorporates these ingredients (animal chows). These high fat food products are considered to be very complex matrix because of the high fat content and hydrophobic nature of the OCPs. Eliminating matrix interferences is essential to be able to efficiently analyze OCPs in screening of contaminated food products. Analysis of complex matrix often requires extensive and labor intense sample preparation (GPC and SPE) to extract OCPs of interest at the appropriate concentration, by removing unwanted lipid matrix co-extractives. We demonstrate the benefits of using Enhanced Matrix Removal-Lipid as a novel dispersive cleanup material that dramatically reduces matrix co-extractives while maintaining excellent analytical accuracy and precision without the need for complex sample preparation techniques. The ease of use, time and cost savings, minimal method development, and dramatically cleaner extracts make EMR-Lipid sample cleanup approach an attractive option for laboratories conducting chemical contamination analysis especially from complex fatty matrices.

Keywords: Food Contaminants, GC-MS, Pesticides, Sample Preparation
Application Code: Food Contaminants
Methodology Code: Sampling and Sample Preparation
Quantification of volatile organic compounds (VOCs) in whole human blood is a reliable way to assess exposure to these toxic compounds. Following exposure, both metabolism and excretion can cause rapid elimination of compounds from the body so sample collection is time sensitive. Many blood samples are collected immediately following an exposure event and then stored at refrigerator temperatures prior to analysis. However, there are some compounds that are not stable in whole blood after sample collection into hermetic blood collection tubes, despite their relative stability in water. Of the 44 compounds included in this study, 37 were found to be stable for up to three months. For example, four compounds decrease as much as 5% per day (1,2-dibromoethane, 1,2-dichloroethane, chloroethane, and methyl isobutyl ketone). The compounds that are stable in the long-term include, among others, trihalomethanes, alkanes, and aromatic hydrocarbons. It has long been acknowledged that the time of collection in relationship to the time of exposure can have a dramatic impact on the measured concentrations in blood. When measuring the compounds identified in this study, it is also necessary to consider the time between sample collection and sample analysis in study design. All data for this study was collected by headspace SPME/GC/MS and isotopically labeled internal standards were added at the time of analysis.

Keywords: Bioanalytical, Environmental/Biological Samples, Gas Chromatography/Mass Spectrometry, Volatile
Application Code: Clinical/Toxicology
Methodology Code: Sampling and Sample Preparation
**Abstract Text**

Most analytical techniques have some interferences that hamper the ability to deliver the best possible analytical results. The concept of sample preparation revolves around manipulating samples to minimize sample-related interferences, maintain the analytical equipment in a clean, non-contaminated condition, and oftentimes reach lower detection limits than the native sample state allows. 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.

Sample preparation operations can be inserted into a chromatographic system at several key points. Figure 1 shows offline and inline sample preparation, some requiring manual operation and some requiring extra valving. 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.

We have developed a vial cap containing an ion exchange filter that functions as the sample is flowed thru it by the action of the autosampler. The particular autosampler design has been used for many years with a filter in the vial cap, and now we add ion exchange resin.

One of the most-used sample preparation chemistries in ion chromatography is the use of acid-form cation exchange to either remove metals from sample matrices that can foul suppressors or system components or neutralize high pH samples, both in the analysis of anions. We have added acid-form cation exchange resin to the vial cap filters so that metals can be removed from samples and high pH samples can be automatically neutralized as the sample flows up thru the cap and into the injection loop. This is the first time in-cap sample preparation has been reported for ion chromatography. In this paper we will show the features of this new concept in sample preparation and also key applications.

**Keywords:** Automation, Instrumentation, Ion Chromatography, Sample Preparation

**Application Code:** Environmental

**Methodology Code:** Sampling and Sample Preparation
The AURA™ Personal Air Sampler (PAS) passively collects an 8-hour whole-air sample via vacuum in a 400 mL canister. The sampler was developed to help environmental and occupational health experts monitor for personal exposures to airborne volatile organic compounds (VOCs). The AURA™ PAS was designed as an alternative to diffusive sampling badges and/or active sampling with thermal desorption (TD) tubes; and was engineered to avoid some of the shortcomings associated with said approaches. A field study applying the AURA™ PAS and the most popular competing technologies has been executed. The current presentation will discuss the results and implications of this study.
## Abstract Text

Both canister and on-line sampling are useful techniques for monitoring trace-level vapours in ambient air using thermal desorption (TD). The first approach involves collecting air into a canister followed by off-line TD–GC analysis, while the on-line method involves the transfer of air directly into the TD–GC system for real-time analysis. These methods are useful alternatives to tube-based sampling when the compounds of interest are too volatile to be retained by sorbent tubes at ambient temperature – for example, hydrogen sulfide.

However, major issues arise when using either of these techniques to sample air streams with high humidity. Specifically, lowered sorbent breakthrough volumes and cold-trap ice formation can lead to lower maximum sample volumes and increased method detection limits, while the presence of water in the GC column can cause poor chromatography.

Removal of water from canister and on-line samples is therefore paramount, but existing approaches have major drawbacks. For example, certain polar species and ultra-volatiles can be lost when using Nafion™ dryers, while very volatile compounds can be lost when using trap dry-purging.

This presentation will describe a new approach to water management for canister and on-line sampling, which involves the selective removal of water prior to analyte focusing. This approach allows high sensitivity analysis of polar species, oxygenates and pinenes (as well as all other typical VVOCs and VOCs) in humid environments.

### Keywords
- Environmental/Air
- Gas Chromatography/Mass Spectrometry
- Sample Handling/Automation
- Thermal Desorption

### Application Code
- Environmental

### Methodology Code
- Sampling and Sample Preparation
Extraction and Purification of DNA from Complex Biological Sample Matrices Using Solid-Phase Microextraction Coupled with Real-Time PCR

The determination of extremely small quantities of DNA from complex biological sample matrices represents a significant bottleneck in nucleic acid analysis. Valuable diagnostic information is often provided by subjecting DNA samples to the polymerase chain reaction (PCR) and/or sequencing methods. These sensitive bioanalytical techniques require the input of highly pure nucleic acids, particularly for the detection of extremely small quantities of target DNA from complex biological sample matrices. In this study, polymeric ionic liquid (PIL)-based solid-phase microextraction (SPME) was applied for the extraction and purification of DNA from crude bacterial cell lysate with subsequent quantification by real-time PCR (qPCR) analysis. Using an on-fiber ultraviolet initiated polymerization technique, eight different PIL sorbent coatings were generated and their DNA extraction performance evaluated using qPCR. The PIL sorbent coating featuring halide anions and carboxylic acid groups in the cationic portion exhibited superior DNA extraction capabilities when compared to the other studied PILs and a commercial polyacrylate SPME fiber. Electrostatic interactions as well as an ion-exchange mechanism were identified as the driving forces in DNA extraction by the PIL sorbents. The selectivity of the PIL sorbent coating for DNA was demonstrated in the presence of PCR inhibitors at high concentration, where a quantifiable amount of template DNA was extracted from aqueous samples containing CaCl₂ and FeCl₃. Furthermore, the PIL-based SPME method was successfully applied for the extraction of DNA from crude bacterial cell lysate spiked with 1 pg mL⁻¹ template DNA without requiring the use of organic solvents or centrifugation steps.

Keywords: Extraction, Nucleic Acids, Sampling
Application Code: Bioanalytical
Methodology Code: Sampling and Sample Preparation
This study offers an integrated approach for time weighted average (TWA) concentration determination of analytes with a wide range of physical-chemical properties. Two types of thin film solid phase microextraction (TF-SPME) passive sampler, namely a retracted TF-SPME device using a hydrophilic lipophilic balance (HLB) sorbent and an open bed configuration TF-SPME device with octadecyl silica-based (C18) sorbent were developed and used for monitoring TWA concentrations of selected UV blockers and biocides. Laboratory calibration results indicated that the thin film retracted device using HLB sorbent is suitable to determine TWA concentrations of hydrophilic analytes in water and uptake was linear up to 70 days. In open bed form, a one-calibrant kinetic calibration technique was accomplished by loading Benzophenone 3-d5 as calibrant on the C18 coating to quantify all hydrophobic compounds. The experimental results showed that the one-calibrant kinetic calibration technique can be used for determination of classes of compounds in cases where deuterated counterparts are either not available or expensive. The developed passive samplers were used in field trials which indicated that these devices are suitable for long-term and short-term monitoring of compounds varying in polarity.

**Keywords:** Environmental Analysis, Liquid Chromatography/Mass Spectroscopy, Sample Preparation, SPME

**Application Code:** Environmental

**Methodology Code:** Sampling and Sample Preparation
Solid phase microextraction (SPME) fibers were developed/patented by Janusz Pawliszyn in 1990 and subsequently licensed to Supeclo until 2014. However, SPME technology has largely remained unchanged over the last 26 years and is subject to following significant drawbacks: limited mechanical stability and small phase volumes. The PAL SPME Arrow was developed to overcome the aforementioned shortcomings. SPME Arrows have an outer diameter of 1.1 or 1.5mm. Compared to traditional SPME fiber diameters of ~0.5 mm, SPME Arrows have relatively larger sorption phase surfaces (up to 6x) and volumes (up to 20x). In addition, the arrow-shaped tip facilitates smooth penetration of vial and injector septa, and the Arrow design fully protects the sorptive material, thereby minimizing adverse influences and loss of analytes during transfer processes. The following presentation will provide an evaluation of PAL SPME Arrows and side-by-side comparison with traditional SPME fibers. An application to Method 8260B, which capitalizes on the claimed advantages of SPME Arrows will be presented as well.

Keywords: Environmental, Environmental Analysis, Headspace, SPME
Application Code: Environmental
Methodology Code: Sampling and Sample Preparation
Quality-control of environmental waters is a major priority of government environmental agencies due to the ubiquitous presence of environmental pollutants, such as pesticides. Determination of these pesticides in water samples at low and sub µg L\(^{-1}\) levels is therefore necessary to meet the requirement of the US environmental protection agency (EPA). Liquid-liquid extraction (LLE), a widely accepted method for routine analysis of water samples in accredited contract analytical laboratories, is generally considered a time-consuming and laborious technique, requiring large amounts of toxic organic solvents.

As an alternative to LLE and other exhaustive methods, Solid Phase Microextraction (SPME), allows for solventless sample preparation and simultaneous extraction, pre-concentration and sample clean-up. Different geometries of SPME were developed and in particular Thin Film Microextraction (TFME) provides a larger sorbent volume in addition to an increased surface area-to-volume ratio resulting in faster extraction rates and order(s) of magnitude increases in method sensitivity when compared to the fiber geometry. It is therefore the intent of this study to perform the optimization of a TFME method for the analysis of a broad range of pesticides in surface water samples. In particular, the challenges related to analyses of hydrophobic analytes were addressed and overcame by the optimization of the sampling approach. Polydimethylsiloxane/divinylbenzene (PDMS/DVB) and PDMS/DVB carbon mesh supported membranes were prepared and extensively evaluated from several analytical aspects including stability, lifetime, sensitivity, accuracy and repeatability. All parameters affecting the extraction, were carefully optimized and the method developed provided limits of detection in the low ng L\(^{-1}\) range, a wide linear range of 0.025-10.0 µg L\(^{-1}\) with strong correlation to response (R\(^2\) = 0.99) and satisfactory accuracy values by both membrane morphologies tested.
Deposition of a Sorbent into a Recession on a Solid Support Provides a New, Mechanically Robust Solid Phase Micro-Extraction Device

Traditional solid-phase microextraction fibers have been limited in their ease of use during in-vivo samplings due to the requirement for a sheathing needle to pre-puncture sample matrices. To address this limitation, a new SPME device is herein proposed which incorporates a coated region which is recessed into a solid support. As the coating surface is flush with, or lies below that of the solid support, the SPME coating does not directly experience the forces associated with the puncture of a sample matrix. The implementation of a recessed extractive coating has been verified as mechanically robust after being incorporated into a projectile which was fired from an air gun into whole fish resulting in no mechanical damage to the coating. In addition, the act of puncturing pre-slit septa in order to access a sample solution was verified to not affect extraction of analyte in a targeted study of pharmaceuticals in water at 15ppb. Next, the recessed SPME device was used for an in-vivo application where raw whole salmon steaks were directly sampled by pushing the recessed SPME device through the protective outer skin in order to sample the underlying muscle tissue for poly-unsaturated fatty acids. Method responses for these compounds were 3-4 times greater than commercially available mixed mode SPME fibers when a hydrophilic-lipophilic balanced coating was used. Certainly, the implementation of the presented device which requires no sheathing needle while being able to directly puncture tissue will provide minimally invasive in-vivo sampling opportunities for a wide range of applications.

Keywords: Bioanalytical, Liquid Chromatography/Mass Spectroscopy, Sampling, SPME
Application Code: Bioanalytical
Methodology Code: Sampling and Sample Preparation
Solid phase microextraction (SPME) is a sample preparation technology that has been gaining wide acceptance in the bioanalytical field. Several studies where either fibres or thin-film SPME devices are employed for the analysis of different biofluids (e.g. urine, plasma and blood) have been recently reported. Considering that in SPME extraction happens via free analyte concentration, it is important to understand how different matrix components could influence the extraction process. In this work, an evaluation of the hematocrit effect on SPME extractions is presented. As a matter of fact, the fraction of blood volume constituted by red blood cells is what is known as hematocrit, and its typical values are within the ranges of 0.41 - 0.50 for men, and 0.36 - 0.44 for women. For this study, thin-film HLB devices and mixed mode SPME fibres of 1.5 cm were used. In addition, three different hematocrit levels were investigated: 0.2, 0.45, and 0.7. As model analytes, drugs of different polarities and moieties were selected. Results showed that, depending on the compound characteristics, increasing, decreasing or no differences in the amount extracted with increasing hematocrit levels can occur. This can be explained considering that SPME is able to extract only an amount of analyte proportional to its free concentration. Therefore, drug affinity toward red blood cells and plasma components determines the final amount available to be extracted by the SPME device. Nonetheless, using an appropriate compound as internal standard provides an effective way to correct for hematocrit level variations.

Keywords: Bioanalytical, Biological Samples, Clinical/Toxicology, Sample Preparation
Application Code: Bioanalytical
Methodology Code: Sampling and Sample Preparation
The research focused on the development of new sorbents for Solid Phase Microextraction (SPME) has been active in the past decade with the purpose of providing extraction phases suitable to a wide variety of applications. In particular, considering the recent advances of the technique in metabolomics and untargeted analysis, the need for coatings able to extract a broad range of analytes especially from complex matrices it is of utmost importance [1]. In this work we present a new generation of biocompatible SPME coatings constituted of HLB particles immobilized by a fluoropolymer [2]. The main merits of this coating are its suitability for both thermal and solvent desorption as well as its compatibility with complex matrices. The extraction performances of the new HLB/F-polymer coatings were compared to conventional coatings previously used for metabolomics investigation by gas- and liquid chromatography using fruit metabolites and drugs bearing large ranges of physicochemical properties. Moreover, the biocompatibility of the new coatings was investigated using direct immersion extraction from complex matrices such as blood, serum, urine, saliva and grape juice. It has been found that the new HLB/F-polymer coatings is compatible with all tested matrices without any biofouling and can be used without loss of performance at least for 50 extractions. Our results showed that the new SPME fiber is a promising tool for integration of metabolomics platforms.


Keywords: Environmental/Biological Samples, Mass Spectrometry, Sample Preparation, SPME
Application Code: Bioanalytical
Methodology Code: Sampling and Sample Preparation
Due to their widespread presence in a number of different matrices such as air, water, soils and food the determination of aliphatic primary amines represents an analytical challenge of great interest to the scientific community. In this work solid-phase microextraction (SPME) analysis of short-chain aliphatic amines (C3-C6) in aqueous solutions was carried out by using pentafluorobenzaldehyde (PFBAY) as on-fiber derivatization reagent. A standard gas generating vial was used for on-fiber loading of the derivatization agent so as to avoid the need for its regeneration at each derivatization cycle. Several parameters such as loading time, reaction temperature, and reaction/extraction time were optimized for headspace and direct sampling in aqueous solutions. Three different SPME coating chemistries were tested and their performances compared in order to achieve the best compromise between sensitivity and analysis throughput. The newly developed PDMS/DVB/PDMS coating showed superior performance in terms of extraction efficiency in addition to the capability to prevent on-fiber degradation of the derivatizing products. The optimized method was used for quantitation of short-chain aliphatic amines in aqueous samples and provided detection limits in the low ppb range for all the amines tested with accuracy values between 79 and 120%. The method was applied towards the analysis of environmental water samples and the accuracy of the results was evaluated by different calibration approaches.

**Abstract Text**

**Keywords:** Adsorption, Derivatization, Environmental Analysis, SPME

**Application Code:** Environmental

**Methodology Code:** Sampling and Sample Preparation
In this study, a polymer-based HILIC column, packed with polyvinyl alcohol base material modified with tertiary amino groups, was used to develop a highly-sensitive LC/MS method to quantify saccharides, organic acids, and amino acids. In order to simplify the conventional analytical method required for derivatization or ion pair reagent, a new LC/MS method was studied using Shodex VG-50.

The authors found that this column, with an aqueous solution including ammonia / acetonitrile as the eluent, was suitable for this analysis. An alkaline condition (max. pH13) is available for this column. Furthermore, the flow rate was suitable for LC/MS analysis, and ESI-MS was used for detection.

It was demonstrated that this method was applicable for the simultaneous analysis of a commercial energy drink separating fructose, glucose, sucrose, citric acid, isoleucine, phenylalanine, threonine, and glutamic acid in a single run.

This HILIC column was found useful for the LC/MS analysis of various hydrophilic substances using more simple conditions than previous methods allowing the analysis of saccharides, organic acids, and amino acids simultaneously using a single column.

Keywords: Amino Acids, Carbohydrates, Liquid Chromatography/Mass Spectroscopy, Spectroscopy

Application Code: Bioanalytical
Methodology Code: Liquid Chromatography/Mass Spectrometry
Analysis of Antibody Drug Conjugates (ADC) by 2 µm Size Exclusion Chromatography Column with Dual Functionality

The monoclonal antibody (mAb) drug market continues to expand. Antibodies, when conjugated with cell-killing cytotoxic drugs, are called antibody drug conjugates. Antibody drug conjugates (ADC) market is expected to be worth more than USD 600 million with very strong potential to grow.

Size exclusion chromatography (SEC) is widely used for the purification of mAb and their conjugates such as ADCs during polishing step. SEC is also used for routine quality control (QC) type analysis. On-line SEC-MS is a useful method to establish Drug antibody ratio (DAR). An ADC (Trastuzumab-vcMMAE) in which an antineoplastic drug (monomethyl auristatin E, MMAE) bonded via a linker to Trastuzumab was used for this study.

TSKgel UP-SW3000 column packed with 2 µm silica-based beads shielded with a hydrophilic diol-type bonded phase is used for this study. This column with dual functionality for its use in both conventional HPLC and UHPLC was used for the analysis of Trastuzumab and corresponding ADC, minimizing the time consuming method transfer related steps. Also online SEC-MS of Trastuzumab and corresponding ADC is reported. Reproducibility of the peak parameters such as retention time (RT), peak area, peak asymmetry, column efficiency in the analysis of Trastuzumab and corresponding ADC is also reported.

Keywords: Bioanalytical, Biopharmaceutical, HPLC Columns, Mass Spectrometry
Application Code: Bioanalytical
Methodology Code: Liquid Chromatography/Mass Spectrometry
Avobenzone is a commonly used organic UV-filter present in 3 to 10% of personal care products worldwide, especially in sunscreen products. To assess the extent of human exposure to avobenzone from use of personal care products, identification of specific biomarkers of avobenzone is required. We investigated the in vitro metabolism of avobenzone using pooled human liver microsomes by online solid phase extraction coupled to liquid chromatography-mass spectrometry. Multiple specific metabolites of avobenzone were chromatographically separated and detected. High resolution mass spectrometry was used to confirm the identity of the metabolites and their isomers. To evaluate the possible usefulness of newly identified in-vitro avobenzone metabolites such as hydroxy- and carboxy- avobenzone as exposure biomarkers, we analyzed human urine samples for these metabolites. Detection frequency and median concentrations of the measured avobenzone biomarkers in urine will be presented. Our results suggest that with appropriate mass spectrometry quantification techniques, exposure to avobenzone may be assessed using these specific biomarkers.
Nutrient restriction, also known as caloric restriction, has been extensively examined for its positive impact on lifespan, immune system boost, and aging. Nutrient restriction, defined as providing essential nutrients but restricting total caloric intake, has shown to decrease disease rate in humans. In addition, nutrient restriction is implicated in decreasing cancer initiation and progression. We have previously established three dimensional cell culture models, known as spheroids, in the HCT 116 colorectal cancer cell line as a high throughput model for studying the proteomic changes associated with nutrient restriction. We found that SUMOylated proteins are up regulated in colorectal cancer 3D cell cultures when subjected to metabolic stress. The attachment of the SUMO protein leads to changes in subcellular location, DNA repair, transcriptional regulation, and mitosis. We will explore SUMOylated in response to nutrient restriction via a novel technique to detect biomolecules using LC-SERS. This method allows the detection of chemical differences associated with biomolecule structure. We first will enhance this system to detect and quantify metabolites in a more complex matrix, such as a cell lysate. Once the methodology has been optimized we use LC-SERS to identify and quantitate SUMOylated proteins in response to NR. When compared to available MS methods to identified SUMOylated proteins, we hypothesize our novel LC-SERS methodology will be able to surpass the limitations found with MS methods. Nutrient restriction has overwhelming effects on cancer and this study will provides further evidence that SUMOylation, in response to metabolic stress, has a role in cancer progression.

Keywords: Bioanalytical, Liquid Chromatography/Mass Spectroscopy, Surface Enhanced Raman Spectroscopy
Application Code: Bioanalytical
Methodology Code: Liquid Chromatography/Mass Spectrometry
Neurotransmitters are essential to cognitive thoughts and behaviors in humans and are metabolically related and affected by external factors. While the enzymatic pathways have been discovered, a large amount remains unknown about the interplay between environment and neurotransmitter metabolism especially concerning the effects from drugs of abuse like alcohol. Successful efforts to quantify this metabolism have used liquid chromatography or capillary electrophoresis with electrochemical detection, UV-Vis, and/or mass spectrometry. Non-mammal model systems have become a popular alternative to mammals for this research due to the smaller costs, less regulations, and simpler upkeep. This study optimized a sample preparation and analysis protocol for non-mammalian research with [i]Drosophila melanogaster[/i] that enabled quantification of neurotransmitters and their metabolites using HPLC-EC. Two known buffer systems, MDTM and citrate/acetate, were adapted, augmented for the separation of 20+ neurotransmitters and metabolites, and subsequently compared using biological samples and standards. The phosphate buffer, MDTM, displayed a more stable baseline current and less noise compared to citrate-acetate, but the reducing potential necessary for analyte detection approached the reduction potential of the phosphate. The citrate acetate buffer provided an increased sensitivity to many of the neurotransmitters and metabolites and included hard to electrochemically detect compounds such as octopamine and all of the N-acetylated catecholamines. This system produced more background noise, but the increase in signal enabled a more sensitive analysis of small biological samples. The use of the modified citrate acetate buffer in future studies of other non-mammalian systems with longer lifespans could provide a more comprehensive picture of neurotransmission and neurochemical metabolism.

Keywords: Bioanalytical, Electrochemistry, Liquid Chromatography
Application Code: Bioanalytical
Methodology Code: Liquid Chromatography
In Vivo Quantification of Melanocortin Peptides Using Capillary Liquid Chromatography-Tandem Mass Spectrometry

Neuropeptides are a complex and functionally diverse group of neurochemical messengers that serve many functions throughout the central and peripheral nervous systems. Challenges facing traditional neuropeptide measurement methods include low peptide extracellular concentrations, inability to measure concentration dynamics, and lack of sequence specificity. Microdialysis enables direct sampling of neurochemicals while removing large proteins based on the membrane’s molecular weight cut-off. Dialysate fractions can then be analyzed using capillary liquid chromatography-mass spectrometry (cLC-MSn), allowing for sequence identification and quantification. The goal of this project is to develop a method to quantify neuropeptide transmission in the central melanocortin system, a circuit involved in controlling energy homeostasis. The instrumental setup utilized a dual-pump, dual valve system for rapid loading and desalting of dialysate samples. Samples are loaded onto a 3.5 cm, 50 μm I.D. capillary column packed with 5 μm diameter particles. Once loading and desalting is complete, a binary pump is switched online and a gradient is run to elute three peptides of interest: melanocyte stimulating hormone (α-MSH), α-MSH(1-12), and [Des-acetyl] α-MSH. The cLC system is directly interfaced to a linear ion trap mass spectrometer operated in MS/MS mode. The high sensitivity of cLC and the sequence specificity of tandem-MS enables reliable separation and quantification of melanocortin peptides in the 1-5 pM range in in vivo dialysate collected from various regions in a mouse model.

This work was supported by NIH Grant # R01DK066604

Keywords: Capillary LC, HPLC, Liquid Chromatography/Mass Spectroscopy, Neurochemistry

Application Code: Bioanalytical

Methodology Code: Liquid Chromatography/Mass Spectrometry
Bioanalytical - MS, MS/GC, and LC/MS

Analyzing Liposomal Drug Delivery Systems in Three-Dimensional Cell Culture Models Using MALDI-Imaging Mass Spectrometry

One of the biggest challenges in current chemotherapy regimens is the failure of active drug to reach all diseased cells. To help solve this problem, researchers are investigating novel drug delivery systems. Liposomes are an attractive option due to their low toxicity and high biocompatibility. This study evaluates the penetration of liposomes encasing doxorubicin, a common chemotherapy drug, into three-dimensional cell cultures, or spheroids. Liposomes composed of phosphatidylcholine (PC) and phosphatidylethanolamine (PE) are created using an extruder. During this process, doxorubicin becomes encapsulated in the hydrophilic core of the liposome. Spheroids were then dosed with liposomes for 24 hours before being cryosectioned for imaging. Doxorubicin is actively released in the spheroid as PC and PE bind cellular lipid bilayers. Drug penetration was visualized using both fluorescence microscopy and Matrix Assisted Laser Desorption Ionization Imaging Mass Spectrometry (MALDI-IMS). These methods are complementary as MALDI-IMS generates ion maps that determine doxorubicin distribution as well as additional analytes, which cannot be determined with fluorescence microscopy. Additionally, drug delivery using liposomes with doxorubicin show an increase in the amount of drug located within the core of the spheroid in comparison to free drug studies. This result indicates better drug delivery to all cells present in the spheroid. This new methodology will help evaluate treatment options, potentially leading to a better treatment in the future.

Keywords: Drugs, Imaging, Lipids, Mass Spectrometry
Application Code: Bioanalytical
Methodology Code: Mass Spectrometry
Dioscoreophyllum cumminsii, a multipurpose medicinal plant is employed in the management of impotence, male infertility, diabetes, obesity and debility. The aim of the study is to relate the constituents of the essential oil with its medicinal applications. The dried and pulverized whole plant was extracted by hydro distillation into hexane. Continuous collection of the essential oil over a four-hour period and hourly collections over four hours were carried out. Analysis was carried out on a gc-ms fitted with HP5MS column using a temperature program of 60 deg. C (2min) increased at 9 deg. / min to 290 deg.C (2min). The major constituents include hexadecanoic acid (1.4-82.7 %), phytol (11.6-19.5 %) in all the samples and 1-(+)-ascorbic acid 2,6-dihexadecanoate (27.3 – 78.9 %) in three of the samples. Phytol has been found to lower serum cholesterol and has been administered to patients with diabetes, obesity and cardiovascular disease which are some of the causes of impotence. Ascorbic acid has been found to enhance sperm motility. It also reduces muscle fatigue. Thus the essential oil contains compounds which are relevant in the management the stated diseases. Funding is by the University of Lagos and the authors.

Keywords: Flavor/Essential Oil, Gas Chromatography/Mass Spectrometry, Natural Products, Semi-Volatiles
Application Code: Bioanalytical
Methodology Code: Gas Chromatography/Mass Spectrometry
Bioanalytical - MS, MS/GC, and LC/MS

GC-MS Identification of the Bioactive Compounds in the Essential Oil from the Aerial Parts of Cardiospermum Halicacabum, and Their Relevance to the Medicinal Uses of the Plant

Cardiospermum halicacabum, a multipurpose medicinal plant, is employed in the management of nervous disorders, headache, rheumatism, hemorrhoids, skin eruptions, syphilitic sores and gonorrhea. The purpose of the study is to identify the constituents of the essential oil which may be relevant in the management of these diseases. The dried and pulverized whole plant was extracted by hydro distillation into hexane. Continuous collection of the essential oil over a four-hour period and hourly collections over four hours were carried out. Analysis was carried out on a gc-ms fitted with HP5MS column using a temperature program of 60 deg. C (2min) increased at 9 deg./min to 290 deg.C (2min). The major constituents include phytol (24.7-67.8 %) and 2-pentadecanone, 6,10,14-trimethyl (25.4-36.2 %) present in all the samples, caryophyllene oxide (19.2-40.1 %) present in the 4-hour and 1st hour samples and hexadecanoic acid (4.4-12.7 %) present in the 4-hour and 4th hour samples. Phytol, caryophyllene oxide and hexadecanoic acid are anti-inflammatory compounds which can be useful in managing rheumatism, hemorrhoids and skin eruptions. Hexadecanoic acid is involved in cell signal and may be relevant in the management of nervous disorder and it functions as an antimicrobial. Caryophyllene oxide functions as peripheral analgesic and exhibits activity against Candida albicans. Thus the essential oil contains compounds which are relevant in the management of the stated diseases.

Funding is by the University of Lagos and the authors.

Abstract Text

Cardiospermum halicacabum, a multipurpose medicinal plant, is employed in the management of nervous disorders, headache, rheumatism, hemorrhoids, skin eruptions, syphilitic sores and gonorrhea. The purpose of the study is to identify the constituents of the essential oil which may be relevant in the management of these diseases. The dried and pulverized whole plant was extracted by hydro distillation into hexane. Continuous collection of the essential oil over a four-hour period and hourly collections over four hours were carried out. Analysis was carried out on a gc-ms fitted with HP5MS column using a temperature program of 60 deg. C (2min) increased at 9 deg./min to 290 deg.C (2min). The major constituents include phytol (24.7-67.8 %) and 2-pentadecanone, 6,10,14-trimethyl (25.4-36.2 %) present in all the samples, caryophyllene oxide (19.2-40.1 %) present in the 4-hour and 1st hour samples and hexadecanoic acid (4.4-12.7 %) present in the 4-hour and 4th hour samples. Phytol, caryophyllene oxide and hexadecanoic acid are anti-inflammatory compounds which can be useful in managing rheumatism, hemorrhoids and skin eruptions. Hexadecanoic acid is involved in cell signal and may be relevant in the management of nervous disorder and it functions as an antimicrobial. Caryophyllene oxide functions as peripheral analgesic and exhibits activity against Candida albicans. Thus the essential oil contains compounds which are relevant in the management of the stated diseases.

Funding is by the University of Lagos and the authors.
Nanocomposite polyvinylpyrrolidone (PVP) fibers were prepared using electrospinning, which is a simple and versatile method to produce polymeric nanofibers. These electrospun polymeric nanofibers were applied as substrates for surface-assisted laser desorption/ionization (SALDI) and matrix-enhanced surface-assisted laser desorption/ionization (ME-SALDI) mass spectrometry. SALDI approach is able to yield high quality mass spectra without interference from the organic matrix, which usually limits the analysis for small molecules (Mw<1,000 Da) and leads to formation of “sweet spot” with poor reproducibility. The electrospun nanofibrous mat typically has high surface area and is promising as SALDI substrates. In this study, polymeric PVP nanofibers were fabricated together with functional inorganic nanoparticles, which could be evenly and firmly immobilized into the nanofibers network. Application of the composite nanofibers as SALDI substrates was evaluated through the analysis of both small molecules and macromolecules, covering a wide range of molecular weights. The ionization efficiency could be improved, resulting in high signal-to-noise ratios for test compounds. And, the SALDI mass spectra showed clean background and enhanced shot-to-shot reproducibility relative to matrix-assisted laser desorption/ionization (MALDI).

Keywords: Mass Spectrometry, Nanotechnology
Application Code: Bioanalytical
Methodology Code: Mass Spectrometry
Analysis of single cells is an important aspect of many disciplines, such as toxicology and medical diagnosis, in addition to drug and cancer research. Due to the ability to simultaneously evaluate large numbers of metal isotopes with high sensitivity and large dynamic range, inductively coupled plasma mass spectrometry (ICP-MS) has become a popular research tool in the fast growing field of high dimensional single cell analysis. Mass cytometry combines ICP-MS with time of flight mass spectrometry (TOF) to determine the properties of a cell. In this technique, antibodies are conjugated with heavy metal ion tags and used to label samples of cells, which can then be analyzed by ICP-MS. The high dimensional analyses offered by mass cytometry permit a detailed evaluation of the phenotypic and functional heterogeneity of cellular samples. However, mass cytometry continues to face challenges in the efficiency of cellular transport to the ICP, which can be particularly problematic when analyzing rare cell populations in limited samples. Our research addresses factors that can affect the condition of the cell prior to introduction to the ICP and ways to improve the efficiency and reliability of sample transport in mass cytometry. A newly designed high efficiency concentric nebulizer with a zero dead volume capillary connection is evaluated in addition to a tool to accurately monitor sample delivery.

Keywords: Atomic Spectroscopy, Bioanalytical, Mass Spectrometry, Plasma Emission (ICP/MIP/DCP/etc.)
Application Code: Bioanalytical
Methodology Code: Atomic Spectroscopy/Elemental Analysis
The methanobactin (mb-OB3b) from [i]Methylosinus trichosporium OB3b[/i] (1154.26 Da) is a member of a class of metal binding peptides identified in methanotrophic bacteria. Methanobactins will selectively bind and reduce Cu(II) to Cu(I), and are thought to mediate the acquisition of the copper cofactor for the enzyme methane monooxygenase. Methanobactins will also bind other metal ions and mediate their solubilization and transportation in situ. The structure of mb-OB3b consists of nine amino acids and two modified regions containing enethiol-oxazolone rings which are the bidentate binding sites for Cu(I). However, the binding sites for other metal ions have not been determined and the goal of this study is to determine whether mb-OB3b binds other biologically active metals in a different coordination than Cu(I). Results of the following experiments will be shown: (1) mb-OB3b titrations using ion mobility mass spectrometry with different metal ions to determine the relative binding affinity for each metal ion; (2) monitor changes in free and metal-bound species as a function of pH; (3) monitor the changes in collision cross section of metal replacement in the mb-OB3b complex; (4) use collision-induced dissociation to determination whether there are differences in the binding of the metals coordination; and (5) repeating titration studies using fluorescence spectroscopy to compare gas-phase and solution-phase techniques.

Keywords: Bioanalytical, Fluorescence, Mass Spectrometry, Metals

Application Code: Bioanalytical

Methodology Code: Mass Spectrometry
One of the largest challenges in science is finding appropriate and reliable model systems. If a microbiologist is tasked with studying interactions in microbial communities, there are few methods for studying more than one or two microbes at a time, which presents unique challenges. One novel and effective way of studying bacterial and fungal relationships was recently described using cheese. Cheese rinds are biofilms, surfaces of adhered microbial cells that bacteria have developed as an adaptation to living in communities with one another. Although various cheeses and rinds exist throughout the world, individual types of rinds contain highly conserved patterns in microbial community composition and thus provide a simple and easily reproducible model for the study of bacterial interactions. The significance of these interactions extends into human health when those communities are found in the foods we consume. In fact, it has been shown that diets rich in dairy do indeed modify the human gut microbiome. To fully understand this phenomenon, however, we must uncover the underlying biochemical reactions. For this study, we investigate the relationships in natural cheese rinds using strains of [i]Staphylococcus[/i] and [i]Brevibacterium[/i]. Specifically, we want to study the molecular signaling produced by [i]Brevibacterium[/i] in response to the presence of [i]Staphylococcus[/i] communities. Cheese curd media can be used to grow large volumes of co-cultures in order to extract and identify an important signaling biomolecule produced by [i]Brevibacterium[/i]. Chemical extractions followed by isolation of the signaling molecule will allow for subsequent analysis using MALDI-TOF mass spectrometry, MS[^n] experiments, and NMR to reveal the amino acid sequence. Upon completion, this study will take a complex food-based microbiome and partially uncover the fundamental biomolecular signaling, providing greater understanding of microbial interactions.

**Keywords:** HPLC, Mass Spectrometry, Natural Products, Tandem Mass Spec

**Application Code:** Bioanalytical

**Methodology Code:** Mass Spectrometry
Phosphorylation is a dominant form of post-translational modification of proteins, involved in numerous biochemical processes such as signal transduction, activation and deactivation of proteins. Determination of the phosphorylation sites of Human Immunodeficiency Virus (HIV-1) proteins could provide insights into how the virus functions in the human host cell, and furthermore develop strategies to reduce its efficacy. Phosphorylation predominantly occurs at the amino acids serine, threonine and tyrosine. Using the sequenced genomes for HIV-1 clades, bioinformatics software (eg.NetPhosK, KinasePhos and GPS) have computed candidate sites for phosphorylation within each protein. Synthetic peptides of these proteins have been placed in environments to induce phosphorylation and later analyzed by mass spectrometry (MS) to validate the bioinformatics data.

In this work, Matrix Assisted Laser Desorption Ionization Linear Trap Quadrupole (MALDI LTQ) is used to analyze phosphorylation. A peptide sequence of a p24 HIV-1 protein has been found to be phosphorylated by a specific enzyme. This study was expanded to the whole p24 protein, where phosphorylation was measured to be in a phosphorylated to unphosphorylated ratio of 1:1000. The goal of this study is to identify potential phosphorylation sites of p24 on the peptide level and to compare the results with both the bioinformatics and p24 whole protein phosphorylation data.

The following work was supported by Professional Staff Congress-City University of New York.
Bioanalytical - MS, MS/GC, and LC/MS

NOBCChE Poster Awardee - Variation in the Extent of Ion Fragmentation in Traveling Wave Ion Mobility Mass Spectrometry

Ion mobility spectrometry is an analytical technique that separates molecular ions in gas phase according to their size, shape and charge states. Ions travel at a velocity inversely proportional to their collisional cross section (CCS) which is a parameter describing the orientational average of collision rates between the ions and the buffer gas molecules, thus giving an insight into the molecular structure of the ions of interest. Besides identifying the shapes of ions, the coupling of ion mobility to mass spectrometry generates a synergistic outcome that enables separation of isobaric ions. The millisecond time for ion mobility separation makes it easy to be hyphenated with LC separation (mins) and mass filtering (μs).

Ions are heated at the desolvation stage in ESI or during the laser ablation in MALDI. Since ion mobility separation occurs under certain electric fields, ions are further heated. One of the commercially available instruments using this technique and widely used in research is Waters Synapt G2 which employs Triwave technology. Besides ion mobility separation, tandem mass spectrometry in either of the collision induced dissociation (CID) cells i.e. Trap and Transfer which sandwich the ion mobility (IM) cell, is possible. In this instrument, a helium gas flow cell is inserted just before the IM cell and it has been reported that the gas cools down the molecular ions. The extent of cooling by the helium gas is not known in the literature. In this study, using 10[μ]M deoxycytidine monophosphate (dCMP) and 1[μ]M berberine as molecular probe ions, we determined the extent of ion cooling as they traverse the ion mobility cell. The findings from this study have implications on the future applications of the Traveling wave technology as well as the ongoing efforts to further develop the ion mobility technique.

Keywords: Bioanalytical, Ion Chromatography, Mass Spectrometry, Tandem Mass Spec
Application Code: Bioanalytical
Methodology Code: Gas Chromatography/Mass Spectrometry
Ionic liquids (ILs) are low melting point organic salts typically composed of a combination of an organic cation and an organic or inorganic anion. In recent years, they have gained extensive attention due to their unique and attractive chemical and physical properties. To date, applications of ILs have been merged into many research areas such as organic synthesis, solid-phase microextraction, electrochemistry and material science. However, deposition of ILs on solid surfaces for various applications is a challenge due to their tendency to flow. Therefore, different types of immobilization methods have been studied to improve the stability of IL coatings.

Herein, imidazolium-based ILs with varying R groups such as methyl-, vinyl- and benzyl- were covalently bonded to pyrrole via an N-substituted alkyl linkage to make pyrrole-based IL monomer units. Electropolymerization of hexafluorophosphate (PF6-) analogues of these monomers were then carried out at constant potential (vs. Ag/AgCl reference) on different types of electrode materials to form N-substituted polypyrrole IL-coated electrodes. After preliminary characterization, the properties of each fabricated electrode were evaluated for their electroanalytical and extraction applications.

**Keywords:** Chemically Modified Electrodes, Detection, Electrochemistry, SPME

**Application Code:** General Interest

**Methodology Code:** Electrochemistry
With one of the widest potential windows of any solid electrode material, boron doped diamond (BDD) has proven excellence as an electrochemical tool. From water treatment and chemical synthesis to electrochemical analysis and sensing, BDD has applications in a variety of sample media. This combined with its stability, low background current, and low double layer capacitance make BDD specifically useful in trace detection, such as the determination of toxic heavy metals and polycyclic aromatic hydrocarbons (PAHs) in water. We have developed several BDD electrode technologies, each tailored to a specific electroanalytical application. With a Nafion coated BDD optically transparent electrode (OTE) we engineered a fluorescence based spectroelectrochemical sensor for a common PAH, 1-hydroxypyrene, achieving a detection limit of 80 nM (17 ppb). With this, we fabricated BDD microelectrode arrays (MEAs) for the detection of toxic heavy metal ions where we obtained parts-per-trillion (ppt) detection limits for lead (Pb) using measurements taking no longer than 2 minutes. Lastly, we also designed and produced BDD rod electrodes for general electroanalytical applications. These BDD rod electrodes exhibit superb electrochemical redox processes where we observe dpeak separations of 65 mV or less in cyclic voltammetry (CV) of potassium ferricyanide. This work further exemplifies BDD as an excellent electrode material while broadening its sensing applications and electrochemical capability.
Electrochemical Measurement of Vesicular Catecholamine Storage and Release: Understanding the Chemo-Brain

Quantification of vesicular transmitter content is important in order to study mechanisms of neurotransmission and malfunction in disease and yet this it is incredibly difficult to measure these small quantities in the attoliter volume of a single vesicle. Recently, we developed new approaches to characterize the contents of mammalian vesicles isolated or in situ, termed as impact electrochemical cytometry and intracellular impact electrochemical cytometry. In these approaches, nanoscale mammalian vesicles are allowed to adsorb to electrodes and subsequently rupture thereby expelling their contents eliciting an oxidation current that can be used to quantify the catecholamine contents of the vesicles. Combined with exocytotic release measurements with single cell amperometry, it is a powerful technique for understanding neuronal transmission.

Chemotherapy with cis-diamminedichloroplatinum (cisplatin) induces mild cognitive impairment (commonly called chemo brain) including memory loss, difficulty for multi-tasking, difficulty focusing, confusion, etc. Pretreatment of cultured pheochromocytoma (PC12) cells with cisplatin influences cell exocytotic ability but with little or no change of vesicle storage. Single cell amperometry reflects that 2 µM cisplatin treatment increases exocytotic event frequency and event current with shorter duration, whereas 100 µM cisplatin treatment decreases exocytotic event frequency and event current with longer duration. In addition, the stability of the initial fusion pore formed in the lipid membrane during exocytosis is also regulated differentially by different concentrations of cisplatin. This study might help to understand the neurological side effects of cisplatin such as mild cognitive impairment and peripheral neuropathy at the single cell level.

Keywords: Drugs, Electrochemistry, Microelectrode, Neurochemistry
Application Code: Neurochemistry
Methodology Code: Electrochemistry
The transport of ions through the cell membrane is regulated by the channel proteins. The redox species which could precisely modulate physicochemical process in the living cells play an important role to implicate intracellular targets via the concentration diffusion. However, the underlying mechanisms and kinetics during the cell redox signaling process lacks of analytical methods. As an attractive method, the nanopore technique could sensing single biomolecules by biomimicking the pore-forming protein. However, it is hardly to generate and measure the redox species. Here, we show a novel bipolar nanopore (BNP) for the first time to electrochemically in-situ generate and monitor the redox species. In contrast to the previous bipolar studies which yielded the redox reactions at the closed conductive tip of nanopeptites, our presented BNP first in-situ generates and transports the redox species inside its lumen. Both the experiments and simulation results demonstrate there exists a controllable diffusion profile of redox species in the presence of electrochemical polarization. Furthermore, the functionalized gold nanoparticles were involved to visualize the diffusion profile due to their self-assembly properties. Our results showed that the catechol modified BNPs achieved the probing of the NADH in a single human breast cancer MCF-7 cell. Therefore, BNPs make easily fabrication of nanoelectrodes which exhibits good ability for simultaneous, cell-non-invasive, long-term measuring of redox biomolecules in the living cells. Taking the advantages of multi nanopore arrays, BNPs could further achieve the multiplexing detections of electroactive molecules simultaneously in a single cell.

Keywords: Bioanalytical, Electrochemistry, Electrodes, Nanotechnology
Application Code: Bioanalytical
Methodology Code: Electrochemistry
In this presentation we describe the effect of aggregation on the oxidation properties of three different size citrate-coated Au nanoparticles (NPs). The oxidation potential of the Au NPs was monitored by electrostatically attaching the NPs to amine-functionalized indium-tin-oxide (ITO)-coated glass electrodes and performing linear sweep stripping voltammetry on the Au NPs in the presence of complexing bromide ligands. When the Au NPs are attached to the glass/ITO electrode from a solution with pH greater than ~3.0, the 4 nm, 15 nm, and 50 nm diameter citrate-coated Au NPs are well-separated in solution and oxidize at 0.698 V, 0.757 V, and 0.943 V, respectively. This potential difference is due to the negative thermodynamic shift in oxidation potential relative to the bulk metal oxidation potential that is proportional to 1/radius and occurs due to the increasing surface energy with decreasing radius. When the Au NPs are attached to the glass/ITO electrode from solutions with pH below 3.0, the Au NPs aggregate in solution due to neutralization of the citrate stabilizer as indicated by the solution color change from red to purple/blue and by the red shift in the plasmon band in the UV-vis solution spectra. The oxidation potential shifts positive for the aggregated 4 nm and 15 nm diameter Au NPs while the oxidation potential does not shift at all for the 50 nm diameter Au NPs. The magnitude of the shift depends on the extent of aggregation, which was controlled by the specific pH value or the time allowed for aggregation. Scanning electron microscopy (SEM) images confirmed the extent of aggregation and correlated well with the red shift in the UV-vis spectra and the positive oxidation potential shift in the voltammetry for the 4 nm and 15 nm diameter Au NPs. This work is important because it shows that voltammetry can be used to monitor metal NP aggregation, which could find use in sensing, monitoring of catalytic NPs, single particle collision studies, or other applications.

Keywords: Electrochemistry, Electrodes, Metals, Particle Size and Distribution
Application Code: Other
Methodology Code: Electrochemistry
We developed a novel comprehensive luminol-Au-microelectrode electrochemiluminescence system, and achieve the electrochemical visualization of hydrogen peroxide inside one cell. The capillary with a tip opening ~2 μm was coated with gold and the porous layer of polyvinyl chloride/nitrophenyloctyl ether, which was filled with the mixture of luminol and chitosan as the microelectrode. Upon contact with the aqueous hydrogen peroxide, hydrogen peroxide and luminol in contact with the gold layer were oxidized under the positive potential resulting in luminescence for the imaging. Owing to the small diameter of the electrode, the microelectrode tip can be inserted into the cell. When applied certain voltage, the bright luminescence observed at the tip revealed the first electrochemical visualization of intracellular hydrogen peroxide. As compared with the traditional disk electrode including the insertion of a metal wire in the glass capillary and the coating of the luminol layer, this electrode design could bring sufficient luminol for the reaction. The further coupling of oxidase on the microelectrode surface could open the field in the electrochemical imaging of intracellular biomolecules at single cells, which benefited the single cell electrochemical analysis.
Electrochemistry

Synthesis and Characterization of Electrospun Iridium-Cobalt Oxide Nanofibers and Their Catalytic Activity for Oxygen Evolution Reaction

In recent years, electrolytic water splitting has been investigated to generate pure hydrogen for sustainable energy system. The anodic half reaction of water splitting, oxygen evolution reaction (OER), is kinetically sluggish reaction with a significant overpotential, which lowers the overall efficiency of water splitting. Iridium dioxide (IrO$_2$) is utilized as one of the most efficient electrocatalysts for the OER in basic media. However, iridium (Ir) is quite expensive and scarce element, and therefore the large scale application is not practical. Cobalt (Co) is nonprecious and has been reported to have relatively good OER activity. In this presentation, we demonstrate the synthesis of one-dimensional (1D) nanomaterials consisted of both Ir and Co mixed oxides (Ir-Co oxides) with the diverse compositions, aiming at developing a cost-effective and highly active OER catalyst. 1D Ir-Co oxides are prepared via simple electrospinning of Ir and Co precursors mixed at different ratios, followed by calcination. The morphologies and compositions of the prepared 1D Ir-Co oxide nanomaterials are characterized by field-emission scanning electron microscopy, transmission electron microscopy, X-ray diffraction and X-ray photoelectron spectroscopy. Electrochemical activities of these nanomaterials for OER are characterized with linear sweep voltammetry. OER activity of 1D Ir-Co oxide nanomaterials is studied systematically as a function of the Ir to Co composition ratios and morphologies.

This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT& Future Planning (2014R1A2A2A05003769).

Keywords: Electrochemistry, Energy, Materials Characterization, Material Science

Application Code: Material Science

Methodology Code: Electrochemistry
An Advanced Set-Up for the Real-Time Determination of Mass-Transfer Parameters During Oxygen Reduction Reaction (ORR) Measurements

For nearly three decades, the polymer electrolyte fuel cell (PEMFC) has competed against other alternative energy solutions for commercial viability as an energy conversion technology. Platinum, as a cathode electro-catalyst material, offers stable over-potential characteristics and relatively fast kinetics for the two electron ORR reaction pathway when applied in acidic media. This makes Pt electro-catalysts ideal candidates for this system. However, the lack of global availability of Pt drives the need for alternative PEMFC catalysts. While a variety of non-platinum materials have been investigated for this purpose, none have reached the actual commercialization stage to date. For the continued development of novel catalyst materials that may eventually replace platinum, it is important to have an experimental set-up that can provide real-time and accurate kinetics data for the ORR along with mechanistic information to gain insight on the reaction pathway.

In this contribution, we demonstrate the use of an advanced electro-catalysis set-up that is tailor-made to investigate electrode kinetics, hydrodynamic mass-transfer effects, and the formation of intermediates in a single run. The investigation is carried out for ORR with platinum and tungsten oxide as a representative example. With dual working electrodes, simultaneous analysis of the ORR and the oxidation of the resulting potential intermediate, peroxide, is carried out using an electronically switchable bi-potentiostat. An advanced software platform facilitates the study of hydrodynamic mass transport through online application of the Levich equation. Using this approach, the calculation of diffusion coefficients, mass transfer rates, and catalytic efficiency is carried out for ORR with tungsten oxide and platinum electro-catalysts.

Keywords: Electrochemistry, Method Development
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Electrochemistry
An Easy-to-Use Low-Noise Nanopore for Controlling Nanoparticle Translocation Dynamics

Nanopores present a unique opportunity for studying single nanoparticles and molecules. Known as the Coulter Principle, the idea has existed for many decades and has moved from the microscale to nanoscale. In an electrolyte solution and under an applied potential there is an ionic current through the nanopore which becomes temporarily blocked by the passage of a nanoparticle, revealing information such as the size and charge of the particles. Current nanopores such as pulled glass capillaries and TEM drilled silicon nitride membranes are prone to clogging and high noise and overly fast translocations. Here we present a new type of nanopore fabricated in a sealed laser pulled quartz capillary using a focused ion beam. This method combines the ease of use and low noise of a pulled glass capillary nanopore with the short and controllable pore length of a drilled silicon nitride membrane. These new nanopores are used to size and count translocating polymeric nanoparticles. The pore length is easily controlled by changing the location of the drilled nanopore on the pulled glass capillary. By changing the pore length we alter the balance of the electroosmotic and electrophoretic forces acting on the translocating nanoparticle and we can tune the translocation time from submillisecond to >10 milliseconds without having to adjust the applied potential, giving another degree of control over the translocation. This work is financially supported by the AFOSR MURI (FA9550-14-1-0003).

Keywords: Characterization, Nanotechnology, Particle Size and Distribution
Application Code: Nanotechnology
Methodology Code: Electrochemistry
Caffeine and vanillin, are two common additives and often coexist in various foods, drinks, and other products. However, excessive levels of CAF and VAN can cause to some undesirable results to their consumers. Too much CAF can usually be treated with no long-term health problems, but this condition can be deadly, especially for younger patients, such as infants and toddlers. The suggested amount of CAF is normally 400 mg per day (equivalent to 4 to 5 cups of coffee) for healthy adults. High CAF consumption can also worsen pre-existing health conditions, such as anxiety. Vanillin is the most important constituent of vanilla flavour. The bulk of the VAN produced was used as a flavouring agent in different foods. The highest amount of VAN can also cause potential damage to human liver and kidney. Therefore, monitoring the levels of these compounds is of great importance.

Poly (Alizarin red S) conducting polymer was prepared on glassy carbon electrode surfaces and the functionalized electrode was used for the simultaneous determination of caffeine (CAF) and vanillin (VAN). The peak currents for the oxidation of both CAF and VAN are increased at poly (alizarin red S) functionalized electrode, which makes it suitable for simultaneous detection of these compounds. The square wave voltammetry peak current of VAN was linear with the concentration of VAN from 0.5 to 250 µM in the presence of 250 µM CAF. The detection limit of VAN was found to be 0.06 µM in the presence of CAF. At the same time, the peak current of CAF was linear with the concentration of CAF from 10 to 450 µM with a detection limit of 0.8 µM (S/N = 3) in the presence of 30 µM VAN. The poly (ARS) functionalized GCE has good reproducibility and high stability. In addition, the proposed method was successfully applied to determine CAF and VAN in energy drink, and vanilla sugar samples with good results.

Keywords: Chemically Modified Electrodes, Method Development, Quality Control, Voltammetry
Application Code: Food Science
Methodology Code: Electrochemistry
Electrochemistry

Analyzing the Bio-Compatibility of Collagen on Electrochemical, Aptamer-Based Sensors

The ability to understand dynamic changes in hormone concentrations [i]in vivo[/i] is a key challenge in advancing medical diagnostics and monitoring. A critical barrier to this type of measurement is the compatible interfacing of inorganic sensors with biology. To achieve this, we have developed and tested nucleic acid aptamer-modified electrode array chips for the direct measurement of the antibiotic Tobramycin (Tob). To impart biocompatibility, I have coupled a collagen-based gel matrix that allows Tob to move through while protecting the sensor and sensing elements from different biofouling components. Specifically, the collagen matrix is hypothesized to inhibit nucleic acid degradation proteins in the blood from reaching the sensor surface. Conversely, uncoated chips become nonfunctional after contact with clinically relevant samples.

Furthermore, I have investigated the effects of collagen film thickness on sensor response time and overall performance. Preliminary conclusions suggest that while the collagen matrix causes a signal response decrease by up to 80%, when the sensor is used to analyze Tob concentration in serum there is a minimal additional response decrease. This indicates that the collagen matrix is adequately coating the sensor, and is allowing the aptamers to bind to Tob without allowing the nucleic acid degradation proteins to penetrate to the aptamers. This newly developed biocompatible hybrid sensor is well suited to perform real-time analysis of hormone release in complex biological media.

Keywords: Biosensors, Membrane, Microelectrode, Voltammetry
Application Code: Bioanalytical
Methodology Code: Electrochemistry
Fast scan cyclic voltammetry (FSCV) is a popular technique for the in vivo measurement of electroactive biological molecules including catecholamines and their metabolites, ascorbic acid, oxygen, and hydrogen peroxide. However, not all analytes of interest are electroactive and thus cannot be directly detected by FSCV. A popular method to detect molecules that are not electroactive is to use an enzyme modified microelectrode to generate an electroactive species through the consumption of the analyte of interest. Chitosan is a natural polysaccharide that can be electrically deposited onto a microelectrode, which entraps enzymes on the surface of the sensor. Chitosan is traditionally electrodeposited onto the surface of a microelectrode through the reduction of protons, inducing a local basic pH shift that causes chitosan to precipitate onto the electrode. Unfortunately, this technique results in the formation of non-uniform films and the formation of bubbles on the film due to hydrogen gas produced during the reduction step. In this work, we show that the simple addition of a redox mediator into the coating solution improves the ability of chitosan to create uniform and reproducible films onto carbon fiber microelectrodes. As a further test, glucose oxidase was embedded into these chitosan coatings to create enzyme-modified microelectrodes capable of detecting glucose. These sensors were characterized analytically, biochemically, and were shown to perform as well as those produced through the reduction of protons. Overall, this work enables the production of more robust enzyme-modified carbon fiber microelectrodes without sacrificing their performance.
Electrochemistry

Fabrication and Electrochemical Characterization of Binary Composites of Iridium and Ruthenium Oxides

Iridium oxide (IrO\(_2\)) has attracted attentions as a promising electrocatalyst in a wide range of electrochemical applications, such as electrochemical water oxidation reaction and chemical sensing (e.g. ascorbic acid sensors, H\(_2\)O\(_2\) sensors, pH sensors, etc) owing to its high catalytic activity, low resistivity, and chemical and thermal stability. Because of its high metallic conductivity and thermodynamic stability, ruthenium oxide (RuO\(_2\)) has been utilized for electrochemical water reduction reaction, oxygen reduction reaction, and biological sensing (insulin, dopamine, NO, H\(_2\)O\(_2\), etc). High capacitance of RuO\(_2\) is also well known allowing its use as a supercapacitor. The mixed materials consisted of both IrO\(_2\) and RuO\(_2\) show good features such as high ohmic conductivity, chemical/thermal stability and enhanced activities in particular toward both electrochemical oxygen evolution and hydrogen evolution reactions. Due to their different chemical properties of iridium and ruthenium, many studies have synthesized these binary composites sequentially. Current study demonstrates two different ways to fabricate IrO\(_2\) and RuO\(_2\) mixed composites (one-pot electrodeposition and one-step electrospinning), and characterizes their electrochemical properties for the utilization of a capacitor, pH sensor, and a biosensor.

Keywords: Bioanalytical, Electrochemistry, Energy, Materials Characterization

Application Code: Material Science

Methodology Code: Electrochemistry
Fast-scan cyclic voltammetry (FSCV) is a novel analytical technique for in vitro and in vivo detection of biologically significant molecules including catecholamines. When used with carbon fiber microelectrodes, FSCV provides micrometer spatial resolution and sub-second temporal resolution while maintaining chemical selectivity and high sensitivity. Carbon fiber microelectrodes can be fabricated in one of two designs: cylindrical electrodes for measurements within three-dimensional environments such as the brain and disk electrodes for measurements upon two-dimensional flat surfaces such as single cells. In this work, carbon fiber disk microelectrodes fabricated with isotropic pitch-based carbon fibers were compared to those fabricated with traditional anisotropic pitch-based P-55 carbon fibers to study the effect of carbon fiber microstructure on electrochemical performance. Each of these two types of electrodes were characterized under various waveforms for a variety of analytes. For each waveform and analyte, the sensitivity, signal to noise ratio, limit of detection, and time response were quantified. These results give insight into the relationship between carbon fiber morphology and electrochemical behavior while also potentially providing an alternative to P-55 based carbon fiber microelectrodes.

Keywords: Bioanalytical, Electrochemistry, Electrode Surfaces, Electrodes
Application Code: Neurochemistry
Methodology Code: Electrochemistry
The quantification of micronutrients, such as magnesium, calcium, potassium, is relevant for identifying health conditions. In particular, potassium levels in blood below 3 mM are indicative of abnormal heart rhythms. Current quantification methods use Ion-selective electrodes, which are fragile, subject to interference from biological samples, often expensive and require calibration and maintenance. We are developing a paper-based device for the electrochemical detection of potassium (figure 1A). Paper-based platforms are useful for point-of-care bioanalysis because of their simplicity, low cost, portability and disposability.

Our assay is based on an unusual electroanalytical method: monitoring the shift in the redox potential due to the concentration of the ion in the sample. For potassium quantification, we quantified the redox reaction of an electrodeposited Prussian blue layer (figure 1B) and observed a proportional shift in the potential of the cathodic peak due to the potassium ion concentration (figure 1C). We successfully tested solutions containing potassium ions in the health relevant range (0-10 mM) on paper-based devices and obtained a reproducible trend correlating the shift in potential to the concentration of potassium (figure 1D). We also observed negligible interference from sodium and magnesium ions. Optimization of the electrodeposition process, testing of human serum samples and assessment of reproducibility and specificity of devices are ongoing. This paper-based device for detection of potassium will represent the first of a collection of solid-state electrochemical micronutrient sensors our group is developing.

Keywords: Bioanalytical, Ion Selective Electrodes, Material Science, Voltammetry
Application Code: Bioanalytical
Methodology Code: Electrochemistry
The catalytic activity of bimetallic particles may vary during the reaction because one metal of the core/shell nanostructure may undergo oxidation or rearrangement by diffusion from the core to the shell or vice-versa due to heat or other reaction condition. Here we describe the use of linear sweep stripping voltammetry (LSSV) in a variety of acid electrolyte solutions to analyze Cu/Au core/shell NPs under a variety of conditions, such as varied Au shell thickness and different temperatures. Citrate-coated 13.5 ± 2 nm diameter bare Cu NPs and Cu/Au NPs with a varied Au shell thickness were synthesized and attached to aminopropyltriethoxysilane (APTES)-modified indium tin oxide-coated glass (glass/ITO/APTES) electrodes. The bare Cu NPs oxidized at -0.1 V vs Ag/AgCl in 10 mM KBr and 0.1 M HClO₄ solution, which is 100 mV negative from bulk Cu, but citrate-coated core/shell Cu/Au NPs displayed oxidation peaks at 0.28 V and 0.75 V (vs Ag/AgCl). The peak at 0.28 V corresponds to Cu, which is shifted positive by the presence of Au, and the peak at 0.75 V is a combination of Cu and Au oxidation, as determined by the peak integrated charge. In this case, the ratio of the two peaks did not equal the mole ratio of Cu and Au used during the synthesis. The peak at 0.28 V is likely due to core Cu oxidation and peak at 0.75 is due to oxidation of the Au shell and Cu coordinated to the Au and Br-. LSSV performed in 10 mM KCl and 0.1 M HClO₄ solution displayed the same peak at 0.28 V along with a second peak at 0.90 V. In this case, the integrated charge ratio of the two peaks equaled the mole ratio of Au and Cu used in the synthesis, confirming that the first peak was solely due to all Cu oxidation and the second peak solely due to Au oxidation. The second peak is more positive since Au oxidation in Cl- is positive of that in Br- and all of the Cu oxidized at 0.28 V due to weak coordination with Cl- compared to Br-.
Electrochemistry

Electrochemical Detection for High Sensitivity Cardiovascular Tests at the Point of Care

A high sensitivity quantitative electrochemical detection platform is being developed for Point of Care (POC) testing. Many recent studies indicated that high sensitivity cardiac troponin (hs-cTn) tests enabled safe rule-out and accurate rule-in of myocardial infarction (MI), thereby facilitating earlier triage. Moreover, the hs-cTn tests appeared to enable prediction of a heart attack within a month of testing and eventual cardiovascular death. Patients considered at low or intermediate risk could be reclassified and receive therapy of known benefit, like Statins and ACE inhibitors, in addition to other behavioral modifications. The platform aims at achieving accurate quantitative results with a fast turnaround time near the patient so doctors can make informed decisions on treatments, reduce the anxiety of patients, and minimize the cost for payers. Here we present results for high sensitivity tests of Troponin I (hs-cTnI), Troponin T (hs-cTnT), and C-Reactive Protein (hs-CRP).

The tests, built on immunoassay techniques, convert targets within a sample to a common mediator compound. The mediator compound is exposed to proprietary electroactive molecules (EAMs), which are subsequently allowed to form self-assembled monolayer (SAM) on an electrode for measurement. The interaction between EAM and the mediator compounds produces a quantifiable change in redox potential of EAMs indicative of the presence of original target. Results can be obtained using traditional electrode interrogation techniques. For the hs-cTnI test, the limit of detection (LOD) is 0.8 ng/L, with the CV% of 6.3% at the 99th percentile (estimated at 10 ng/L). Through Passing-Bablok regression analysis, good correlations with predicate devices were demonstrated with R2 values in the range of 0.9-0.95. LOD for both hs-TnT and hs-CRP tests is 1 ng/L.

Abstract Text

A high sensitivity quantitative electrochemical detection platform is being developed for Point of Care (POC) testing. Many recent studies indicated that high sensitivity cardiac troponin (hs-cTn) tests enabled safe rule-out and accurate rule-in of myocardial infarction (MI), thereby facilitating earlier triage. Moreover, the hs-cTn tests appeared to enable prediction of a heart attack within a month of testing and eventual cardiovascular death. Patients considered at low or intermediate risk could be reclassified and receive therapy of known benefit, like Statins and ACE inhibitors, in addition to other behavioral modifications. The platform aims at achieving accurate quantitative results with a fast turnaround time near the patient so doctors can make informed decisions on treatments, reduce the anxiety of patients, and minimize the cost for payers. Here we present results for high sensitivity tests of Troponin I (hs-cTnI), Troponin T (hs-cTnT), and C-Reactive Protein (hs-CRP).

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Keywords: Clinical Chemistry, Electrodes, Immunoassay, Quantitative

Application Code: Bioanalytical

Methodology Code: Electrochemistry
Electrochemical Deposition of Tantalum in Non-Aqueous Media and Its Electrochemical Applications

Tantalum (Ta) has been used for many applications such as coating and biomedical materials due to its high melting point, toughness, biocompatibility, and corrosion resistance. Despite of plausible applicability of Ta, there has been few report dealing pure Ta as an electrocatalyst rather than Ta-composites. One of the reasons for this, we think, stems from difficulty in electrodeposition of Ta in an aqueous solution because of preceding H\textsubscript{2}O reduction. To circumvent this problem, previous works have used rather sophisticated non-aqueous systems such as ionic liquids and molten salts. In the present study, we have electrodeposited Ta on carbon black loaded electrodes in an aprotic solvent, acetonitrile. Prepared Ta has been investigated to estimate electrocatalytic activities toward reduction of oxygen and hydrogen peroxide, and redox of various biomaterials. It has represented comparable activity toward oxygen reduction reaction to platinum, and decent performance for selective reduction of hydrogen peroxide. In addition, Ta has been also deposited on pencil lead as a low-cost electrode for real-time amperometric measurement of hydrogen peroxide with short response time. It has been tested for hydrogen peroxide related to metabolism, i.e., estimation of catalase activity and kinetics of glucose oxidase reaction.

Keywords: Bioanalytical, Biosensors, Electrochemistry, Material Science
Application Code: General Interest
Methodology Code: Electrochemistry
Epidemiological studies estimate that greater than 60% of the adult US population may be categorized as either overweight or obese. There is a growing appreciation that the complications of obesity extend to the central nervous system (CNS) and result in increased risk for neurological co-morbidities like depressive illness. Given the hypothesized role of serotonin (5-HT) in the pathogenesis of depression, it is possible that decreases in brain 5-HT efflux induces depressive illness in obesity.

We previously demonstrated that rodents with a phenotype that is consistent with features of the metabolic syndrome (MetS) exhibit depressive-like behaviors. To identify the potential underlying mechanistic mediators of these behavioral changes, plasma was analyzed for inflammatory changes, in vivo microdialysis and fast scan cyclic voltammetry were used to determine hippocampal 5-HT levels following intraperitoneal (i.p.) administration of the selective serotonin reuptake inhibitor (SSRI) fluoxetine. Plasma cytokine analysis revealed that the pro-inflammatory cytokines (IL-1 and IL-6) and plasma C-reactive protein levels were increased in obese rats. Neurochemical analyses determined that Hypo-IRAS animals exhibited decreased basal 5-HT and decreased SSRI-stimulated increases in hippocampal 5-HT efflux.

Collectively, these data support studies indicating that obesity and MetS increase the risk for mood disorders and suggest that changes in 5-HT levels may be a shared feature between depressive illness and metabolic disorders. Additionally, results identify pharmacological differences that provide a mechanistic basis for the decreased efficacy of SSRIs in the treatment of depressive illness in obese individuals.

Supported by Department of Veterans Affairs grant numbers 1101 BX001374 (MAW), I21 BX002085 (LPR), IO1 BX001804 (LPR) and the University of South Carolina School of Medicine Research Development Fund (LPR)
DMSO Slows the Second Step of Exocytosis and Changes the Fraction of Partial Catecholamine Release

DMSO is frequently used as a solvent in biological studies and also as a vehicle for drug therapy; therefore it’s important to study the side effects of DMSO especially on the cell environment. In this work we used a novel method, intracellular vesicle electrochemical cytometry, which is developed in our lab recently. A nano-tip conical carbon fiber microelectrode is used to electrochemically measure the total contents of electroactive neurotransmitters from individual nanoscale vesicles in chromaffine cells and compared with exocytosis. Results show by incubating the chromaffin cells with DMSO for 30 min, the neurotransmitter resale, significantly increase while the vesicular contents don’t change significantly. In addition the kinetic of release in DMSO treated cells are faster than non-treated ones. These changes can be the effect of DMSO on cells membrane.
We have performed electrochemical measurements of exocytosis combined with intracellular electrochemical cytometry to evaluate the effect of anticancer drugs on the nervous system at the single cell level. The drug tamoxifen has been used for over 40 years in the treatment of estrogen receptor-positive breast cancers during both early stages of the disease and in the adjuvant setting. However, because drug treatment with tamoxifen is not ideal, other hormonal chemotherapies have been developed. Anastrozole, an aromatase inhibitor, has been widely used in combination with tamoxifen to reduce the risk of disease recurrence in patients. Both tamoxifen and anastrozole cause memory and cognitive dysfunction, but the reasons for the cognitive impairment and memory problems induced by these anti-cancer drugs are unknown.

PC12 cells were treated with different concentrations of tamoxifen, anastrozole, and their mixture for specific periods of time. Single-cell amperometry was subsequently used to monitor individual exocytotic events and catecholamine release. In order to examine how the vesicular neurotransmitter content changes upon drug treatment, intracellular vesicle electrochemical cytometry was carried out by pushing a nanotip carbon-fiber microelectrode through the cell membrane without significant damage and the whole catecholamine content of individual vesicles in live cells was measured.

Testing the anticancer drugs effect on neurotransmitter secretion and storage will help to understand the actions of these drugs in regulation of vesicles and exocytosis and the communication between nerve cells, and importantly, could have implications for the clinical care of the patients that use them long term.

**Keywords:** Biomedical, Drugs, Electrochemistry, Neurochemistry

**Application Code:** Biomedical

**Methodology Code:** Electrochemistry
Single cell amperometry is a real time chemical analysis method that can be used to quantitatively analyze electroactive neurotransmitter released from single cell during exocytosis. We chose to investigate general anesthetics, essential to both medical practice and experimental neuroscience, as they have potent and selective effects on neurotransmission. These effects include both presynaptic actions and postsynaptic actions. The molecular and cellular mechanisms of anesthetic-induced amnesia, unconsciousness, and immobilization are incompletely understood. Knowledge of the fundamental synaptic effects of anesthetics is therefore essential to a molecular and physiological understanding of anesthetic mechanisms, and to development of more selective and safer anesthetics. Barbiturates are among the most extensively studied central nervous system depressants and are commonly used as anesthetic. So, in this work, we choose to study the role of barbiturate in exocytosis.

The pheochromocytoma (PC12) cell is an excellent model for the study of different aspects of neuronal physiology and biochemistry. Therefore, in this work, it was used for investigating the roles and the mechanism of barbiturate on exocytosis. By analyzing the parameters calculated from single exocytosis events, we observe that barbiturate accelerates the event dynamics and significantly decreases the monoamine amount released from single exocytotic events. Moreover, results from intracellular vesicle impact cytometry show that the vesicular content does not change significantly upon barbiturate treatment. Therefore, barbiturate appears to influence exocytosis by altering the fusion pore dynamics during the release event rather than the vesicle properties. This is important as it shows that release dynamics and not only vesicle content are important in transmission.

Keywords: Bioanalytical, Electrochemistry
Application Code: Neurochemistry
Methodology Code: Electrochemistry
A new concept of electrochemical measurement on a semiconductor electrode, referred to as light activated electrochemistry, has been developed. This method combines electrochemistry with light to remove the constraint of requiring a wire to every electroactive element in an array. Light activated electrochemistry switches an insulated surface to a conducted one, which has not been possible with conventional electrochemistry. This is possible as on poorly doped Si (100) electrode, electrochemistry of redox species can only occur if illuminated by visible light. Hence if only a small region on the surface of a silicon electrode is illuminated, then only that region will give an electrochemical signal, while the rest of the surface not irradiated by light, will be electrochemically inactive. The discovery of light activated electrochemistry allows the high-density DNA microelectrode array to be formed and electrochemistry to be performed anywhere on a 30 [micro]m spot size of the Si surface for a defined time. This system is achieved by coating poorly doped silicon semiconductor with a symmetrical 1,8-nonadiyne followed by ‘clicking’ onto the surface single strands of DNA, which acts as probe DNA. Then the modified electrode is exposed to target DNA to hybridize. Afterward, it is exposed to anthraquinone (AQMS) solution as redox species to intercalate with DNA duplex. Then DNA modified Si electrode is illuminated with a focused light and the light tip is scanned along the x- or y-axis direction. Amperometry is used to monitor the electrochemical redox species current.

Keywords: Biosensors, Detection, Microelectrode, Semiconductor
Application Code: Bioanalytical
Methodology Code: Electrochemistry
Conformational fluctuations in molecules (e.g., proteins) play a critical role in electron transfer reactions. Some of the conformational fluctuations are fast and distribute over a broad time range, and measuring them require a fast and conformational sensitive technique. Here we report a plasmonic detection technique with 10 ns response time, and study of electron transfer reaction in cytochrome c adsorbed on a gold electrode with the technique. Our results show that the conformational fluctuations gate the electron transfer reaction of the protein, and the time scale of the gating occurs from ns to ms. The work demonstrates the capability of the combined electrochemical and plasmonic approach for measuring fast electron transfer processes and conformational changes associated with the electron transfer processes.
GC inlet liners can be nearly as important to good chromatography as column selection. Much attention is given to GC columns; their phase selectivity, so-called “Fast GC,” inert and low-bleed varieties. Columns are routinely the primary component discussed when reviewing method development. However, analysts often overlook the much needed, yet forgotten GC inlet liner.

This poster will review the importance of GC liners and demonstrate how given the same GC instrument parameters and column, very different chromatographic responses can be achieved by using different liners.

Keywords: Chromatography, Gas Chromatography, GC, Optimization
Application Code: General Interest
Methodology Code: Gas Chromatography
Polycyclic aromatic hydrocarbons (PAHs) are important environmental pollutants originating from a wide variety of natural and anthropogenic sources. PAHs are generally formed during incomplete combustion of organic matter containing carbon and hydrogen. Due to the carcinogenic nature of some PAHs, the measurement of PAHs is of great environmental and toxicological importance. Among the hundreds of PAHs present in the environment, the U.S. Environmental Protection Agency (EPA) have included sixteen in their priority pollutants list with molecular mass (MM) ranging from 128 Da to 278 Da. The PAHs included in the EPA list only include a limited number of isomeric PAHs with MM 228 Da (2 isomers), 252 Da (3 isomers), 276 Da (2 isomers), and 278 Da (1 isomer). The National Institute of Standards and Technology has issued SRM 2260a, a mixture of 22 PAHs that include isomers of MM 228 Da (4 isomers), 252 Da (7 isomers), 276 Da (3 isomers), 278 Da (5 isomers), and 302 Da (3 isomers). SRM 2260a is intended for use in the calibration of chromatographic instrumentation and for column evaluation. In the present study, the selectivity differences among three different gas chromatography PAH columns are investigated: SLB-PAHms (50% phenyl-dimethylpolysiloxane phase), SLB-ILPAH (ionic liquid phase), and LC-50 (liquid crystalline phase).
In this study, we will demonstrate how simple modifications to a standard GC can significantly reduce analysis times. Gas Chromatography is one of the most widely used analytical techniques in the world, yet most analysis times are measured in minutes rather than seconds. Small column diameters and thin films can be used in conjunction with hydrogen carrier gas to produce flatter Van Deemter curves (maintaining higher efficiencies over a wider range of flow rates). Using the Van Deemter equation as a guide, we investigate the best combinations of ultra-short (<1M), small diameter (50 μm), thin film columns with high flow rate hydrogen carrier gas and fast heating. The work was done on a modified Agilent 6850 GC with a high voltage (240V) power supply using hydrogen as a carrier gas. The results include a 10 second temperature programmed run of a 10-component hydrocarbon/alcohol mix. We also investigate reducing injection-to-injection times through the use of a gas sampling valve as opposed to a traditional liquid autosampler. The combination of ultra-fast analysis and automated sampling valves also opens up the possibilities of converting a benchtop GC to an on-line analyzer.
ASTM Method D2887 is a simulated distillation method used to determine the boiling point ranges of products ranging from 55.5 to 538°C. One of the applications of this standard method is to demonstrate compliance to Jet Fuel and Diesel Fuel specifications. It also ensures the product conforms to the correct boiling point range during production. Many industries rely upon the importance of the solution this method including the petroleum, chemical, and auto/aviation sectors.

Since there are many samples needing testing, productivity is extremely important. ASTM Method D2887 Option B allows for faster chromatography while maintaining the boiling point accuracy demanded by the industry for this solution.

Using new gas chromatography (GC) oven technology, this poster will demonstrate how to attain faster analysis and enhanced retention time repeatability, optimizing sample throughput while achieving excellent accuracy. Correlation to physical distillation ASTM D86 on “real world” samples will be provided.

Keywords: Fuels\Energy\Petrochemical, Gas Chromatography, Hydrocarbons
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Gas Chromatography
Particle size can play an important role when selecting solid support material. Not only do smaller particle sizes have higher peak efficiencies and thus plate height (H) is reduced, but the optimum linear velocity window at which the minimum plate height is achieved is also increased. This presentation will compare chromatographic performance of three different mesh sizes using a new source of Diatomaceous Earth (DE). Columns packed with mesh sizes of 60/80, 80/100 and 100/120 will be compared using van Deemter plots and normalized efficiency using HETP (height equivalent to a theoretical plate) as a function of mobile phase linear velocity (pressure on column needed to achieve required flow). Inertness of the material compared to current DE solid support will also be presented.
American Society for Testing and Materials (ASTM) Method D8028 is a method to determine dissolved gases in water. The method describes calibration and sampling techniques. One of the recommendations is to ensure the sample is sealed in order to safeguard the integrity of the sample. This recommendation is taken from United States Environmental Protection Agency (USEPA) Method 5030C used for the sampling of volatile organic compounds in water. Many environmental labs currently open their dissolved gas samples in order to place them in a headspace vial for sampling and analysis. This application will compare the efficacy of ensuring the sample is sealed versus opening the sample and pouring it into the sample vial for analysis.

Keywords: Environmental/Water, Fuels\Energy\Petrochemical, Gas Chromatography, Sampling
Application Code: Environmental
Methodology Code: Gas Chromatography
The demand of increased productivity is more and more frequent in modern laboratories along with high reproducibility and repeatability requirements.

In this work two traditional applications, performed with a Dual simultaneous injection technique, are reported. This enables to cope with the market requests.

The first one is the determination of fatty acid methyl esters (FAME) eluting in more than 60 minutes, simultaneously in two different channels (inlet, column, detectors) of the GC At the same time we are able to quantify two different samples, therefore doubling the productivity.

In the second application the capability to increase both selectivity and sensitivity has been highlighted. The GC was configured with two selective detectors (ECD and NPD). This allows different classes of compounds, chlorinated and nitrogen containing compounds, to be determined at the same time.
Introduction.
Phenols are largely present in plants and agro-industrial byproducts, and they represent secondary metabolites in foods and natural health products. The food processing industries generate considerable quantities of various phenolic byproducts. At the same time some simple alkylphenols also exhibit useful properties for the industry. Thus, tert.-butylphenol is an intermediate in production of oil field additives, fragrances and stabilizers; di-tert.-butylphenols are industrially used as stabilizers and antioxidants for fuels and plastics. Identification of alkylphenols and differentiation of isomers in this series by GC-MS can be achieved with an employment of suitable chemical modification methods. A comparative study of trialkylsilyl and perfluoroacyl derivatives of tert.-butylphenols and their thiophenol analogs will be presented.

Results and Discussion.
Comparative analysis of capabilities and limitations of various derivative for structural analysis of phenols and benzenethiols will be presented. Trifluoroacetyl, pentafluoropropionyl and heptafluorobutyryl derivatives will be recommended for structure determination by GC-MS of structural isomers of butylphenols, butylbenzenethiols and butylhydroquinolines. Preparation of trimethylsilyl and tert.-butyldimethylsilyl derivatives prior GC-MS analysis will be advised for the study of alkoxyphenols. Interaction of neighboring functional groups under electron ionization leading to ortho-effects will be presented along with the processes due to para-effect.

Keywords:
Derivatization, Environmental, Food Science, Gas Chromatography/Mass Spectrometry

Application Code: Environmental

Methodology Code: Gas Chromatography/Mass Spectrometry
Over the years, extensive evaluations of columns manufactured with ionic liquid stationary phases have occurred. Their main strength was discovered to be unique selectivity. 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 dicationic phases. Columns prepared with di- or tricationic phases 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 petrochemical, pharmaceutical, environmental, food and beverage and flavor and fragrance.
Over the past 50 years, Gas Chromatography has matured, and largely meets analytical performance demands for the applications it serves. However, overall usability still has not met the expectations and needs of today’s routine GC lab. Reducing unplanned downtime has been a keen imperative of GC enterprises striving to improve business results and reduce business uncertainty. However, chromatographic expertise for maintaining and troubleshooting is no longer always at point of instrument use, necessitating greatly reduced operational complexity. This requires a transformation in the way GC is carried out.

Keywords: Chromatography, GC, GC Columns, GC-MS
Application Code: Other
Methodology Code: Gas Chromatography
Analysis of FAMEs (Fatty Acid Methyl Esters) is commonly conducted using long, highly polar capillary GC columns. Typically, the standard 37 food industry FAMEs are analyzed on 100m long bis-cyanopropyl columns with a run time about one hour. Such columns provide a base-line separation of most peaks but several components are still only partially resolved. We have developed a new, highly selective GC phase for the analysis of FAMEs. The 20m column provides a baseline separation of all 37 standard food industry FAMEs in under 25 minutes.
Owing to their unique physicochemical properties, room temperature ionic liquids are the object of growing interest in separation techniques. The unique properties of modern dicationic ionic liquids such as broad liquid range, high thermal and chemical stability, high viscosity, low volatility, good wetting ability, and the capability of undergoing multiple solvation interactions with a variety of solutes make these type of materials potential candidates for coatings in gas chromatography capillary columns. Herein, we report synthesis of a series of dicationic ionic liquids with structural variation in their linkage chain. Dimethylimidazolium, methylpyrrolidinium and tripropylphosphonium cations were joined via different alkane linkage chains and the resulting dications were paired with bis(trifluoromethane)sulfonamide anion. Alkane linkage chains contain small or bulky groups (methyl, dimethyl, or t-butyl) on one end, both ends or central carbon. Effect of these structural variations on physicochemical properties of these ILs were investigated in detail. Thermal stability of the synthesized ILs was evaluated by TGA. These materials were then coated on capillary columns to examine their thermal stability utilizing inverse GC. Complementary physical characterization of these ionic liquids was performed in terms of their melting points, densities, viscosities and miscibility with polar and nonpolar solvents. Finally, these ILs were used as GC stationary phase to evaluate their selectivity toward different analytes. The results were compared with commercially available GC columns.
High stability ionic liquids (ILs) have a wide range of applications such as gas chromatographic stationary phases, solvents for high temperature reactions, high temperature lubricants, and so forth. Geminal dicationic ILs often display higher thermal stabilities, viscosities, and densities compared to traditional monocationic ILs. Also, their physicochemical properties can be tuned by different structural modifications. So, understanding the effects of different structural modifications on physicochemical properties is important if one wants to introduce or enhance a desired property into an IL. Geminal dicationic ILs can be considered as a combination of three main structural moieties: (1) a cation head group, (2) an alkane linker chain, and (3) the anions. A series of thirty-six geminal dicationic ILs were synthesized and characterized in terms of their physicochemical properties. The effect of each component on the physical and chemical properties was studied by single variable modifications. The synthesized ILs displayed thermal gravimetric analysis stabilities in the range of 330 – 467 °C. Also, nine ILs with high TGA stability and low melting points were tested with inverse gas chromatography (GC), and some of them displayed stabilities up to 400 °C. These nine ILs were further evaluated for performance as the GC stationary phases. The selectivities of these IL stationary phases were compared to each other by separation of different aliphatic and aromatic hydrocarbons, polyaromatic hydrocarbons, and fatty acid methyl esters, etc. The study focuses on understanding the effects of different structural modifications on the physicochemical properties and selectivities of geminal dicationic ILs.

Keywords: Environmental Analysis, Gas Chromatography, GC Columns, Separation Sciences

Application Code: Material Science

Methodology Code: Gas Chromatography
Labeling of proteins for analytical measurements, including those for clinical immunodiagnostic applications, is often accomplished through covalent attachment of the label to the amine side-chains of surface lysine residues. An obvious consideration in protein labeling with these methods is that the protein solution matrix must be free of amine-containing compounds that would compete with the labeling reaction. Unfortunately, several commonly used buffers that are used in purification of these proteins contain free amine groups and must be removed prior to the labeling process. To assure removal of these buffer components we have developed and implemented a quantitative UPLC method to measure residual amounts of glycine and Tris based on Waters AccQ-Tag Amino Acid Analysis (AAA) method. Over the years of applying this method we have occasionally observed additional peaks in the chromatograms with retention times corresponding to no previously identified amine-containing compound, including all common amino acids. Purified protein containing 5% sucrose in the solution matrix yielded four additional unidentified peaks in the UPLC chromatogram. UPLC-MS analysis of these expected impurity peaks curiously all produced an m/z of 513, which matches the added mass of sucrose itself + the AccQ-Tag label. It was concluded that the additional peaks seen in the UPLC analysis of sucrose-containing protein matrices arise from a secondary reaction of the AccQ-Tag reagent with the hydroxyl-groups on sucrose. This secondary reaction should therefore also be considered in any amino acid analysis where a relatively high concentration of hydroxyl-containing compound is present.

Keywords: Bioanalytical, HPLC
Application Code: Bioanalytical
Methodology Code: Liquid Chromatography/Mass Spectrometry
A unique “dilute-and-shoot” method is demonstrated for the analysis of illicit substances mitragynine (e.g. Kratom) and nine synthetic cathinones. Samples were diluted 5X with internal standard solution and analyzed on a TQD LC-MS/MS platform using a 1.7 [mu]m BEH C18, 2.1 x 50 mm column. The cycle time for this method is 2.5 minutes. No sample clean-up or extraction was performed. This assay monitors two transitions for each of the following ten analytes: alpha-PVP, butylone, ethylone, MDPV, mephedrone, methcathinone, methedrone, methylone, mitragynine, and naphyrone; and two internal standards: methylone D3 and alpha-PVP D8. Lowering the column temperature from 50[degree]C to 20[degree]C allowed chromatographic resolution of ethylone and butylone isomers. Daily 3-point calibration curves routinely demonstrated 100 ± 25% accuracy for each point along with excellent curve fits (R[sup]2[/sup][greater than]0.99) and acceptable limits of quantitation/limits of detection (LOQ/LOD = 25 ng/mL). Insignificant matrix effects were observed while interferences were undetected from the tested drugs of abuse (Benzoylcegonine, THC-A, Amphetamine, Methamphetamine, MDMA, MDEA, MDA, and Phentermine), and therapeutic drugs (Oxazepam, Morphine, Imipramine, Buprenorphine, Fentanyl, Meprobamate, Methadone, Tramadol, gabapentin, pregabalin, and tapentadol) at upper limits of linearity. No appreciable carryover was observed following the highest curve point of 1000 ng/mL. By moving away from costly extraction and time-consuming concentration protocols, the utility of this LC-MS/MS dilute-and-shoot method for the analysis of patient urine samples will be demonstrated for clinicians in terms of the reduction of time and labor per sample, conservation of sample, and the preservation of required LOQ/LOD values for diluted samples.

Keywords: Clinical/Toxicology, Drugs, Liquid Chromatography/Mass Spectrosopy, Toxicology
Application Code: Clinical/Toxicology
Methodology Code: Liquid Chromatography/Mass Spectrometry
EISO displayed antimycobacterial and antiplasmodial activities through unknown mechanisms. The goal of this study is to determine whether EISO exerts these by inhibiting enzymatic targets Mycobacterium tuberculosis shikimate kinase (MtSK) and Plasmodium falciparum thioredoxin reductase (PfTrxR) utilizing ESI-QTOF LC-MS-based functional assay approach. MtSK catalyzes the ATP-dependent phosphorylation of shikimate to shikimate-3-phosphate (S3P) in the shikimate pathway responsible for aromatic amino acids biosynthesis. PfTrxR catalyzes reduction of thioredoxin disulfide (Trx-S2) to thioredoxin dithiol (Trx-(SH)2) essential for antioxidant defense of the parasite.

MtSK-inhibitory assay was carried out using a phenyl-hexyl 4.6x100mm,3.5 µm column with a gradient mobile phase of 0.1% formic acid (FA) in water and acetonitrile. Direct inhibition of S3P production was monitored through relative peak areas by LC-MS. EISO at 0.01% v/v in Tween-20 displayed 60% MtSK inhibition with IC50 of 0.015% v/v.

EISO PfTrxR-inhibitory activity was evaluated using a Zorbax 300SB-C8 column, 2.1x100mm,3.5 µm and a gradient mobile phase of 0.1% FA in water and 0.1% FA in acetonitrile. Inhibition of Trx-(SH)2 formation was monitored by LC-MS. To distinguish the two forms of thioredoxin, known major m/z values of multiply-charged protein envelope in the Trx–(SH)2 spectrum were deconvoluted to a product protein at a mass 11675.8711 Da and used for relative quantitation by displaying its extracted ion chromatogram. Major m/z values for the substrate Trx-S2 were deconvoluted at the mass 11673.9204 Da. The 0.01% EISO in Tween-20 showed 90% PfTrxR inhibition.

This study reports LC-MS-based screening of EISO and its inhibitory activity on MtSK and PfTrxR.

Keywords: Enzyme Assays, Flavor/Essential Oil, Liquid Chromatography/Mass Spectroscopy, Protein
Application Code: Drug Discovery
Methodology Code: Liquid Chromatography/Mass Spectrometry
The reliable and deep mapping of human proteome in large sample cohorts is urgently needed to connect genomes and phenotypes, to increase the efficiency of developing new biotherapeutics, and to diagnose or prognose diseases at early stages. The complexity of the human proteome requires highly efficient separation of peptides generated during enzymatic protein digestion and sensitive MS detectors with wide dynamic range in order to find significant differences in abundance of protein species. In this study we combined the new UltiMate™ 3000 RSLCnano ProFlow™ nano LC system with the high-resolution accurate-mass Q Exactive™ HF mass-spectrometer to achieve high analytical depth for human cellproteome profiling. Tryptic peptides were separated on a 75 µm x 75 cm, Acclaim PepMap column. This allows to obtain peak capacity of 800 in 120 minutes and identify more than 5000 protein groups with data-dependent acquisition (DDA). ProFlow technology unlocks high retention time precision that significantly boosts the number of chromatographic peaks that can be aligned between multiple runs and thus, precisely quantified. The deepness of proteome profiling assessed by the range of emPAI extends over 4 orders of magnitude for DDA acquisition and 5 orders of magnitude for Full MS acquisition at 120,000 MS resolution. The obtained results show high analytical precision that ensure accurate label-free quantification and confident selection. This high level of data quality will enable large comparative studies where several thousand proteins can be quantified simultaneously. Ultimately this facilitates confident discovery of regulated peptides in case-control and clinical biomarker studies.

Keywords: HPLC Columns, Liquid Chromatography/Mass Spectroscopy, Peptides, Separation Sciences
Application Code: Genomics, Proteomics and Other 'Omics
Methodology Code: Liquid Chromatography/Mass Spectrometry
Polysorbate 80 as a nonionic surfactant is widely used in the industries of food, cosmetics, personal care, pharmaceuticals and biofuel. It plays an important role as emulsifier, stabilizer and solubilizer. The full chemical name of polysorbate 80 is polyoxyethylene (20) sorbitan monooleate, which is made from polyethoxylated sorbitan and oleic acid. Although polysorbate 80 is chemically named as monoester of polyethoxylated sorbitan and oleic acid, it is actually a mixture of polyoxyethylene sorbitan monooleate (PS), polyoxyethylene sorbitan dioleate (PSD), polyoxyethylene sorbitan trioleate (PSTri), polyoxyethylene sorbitan teraoleate (PSTetra), polyoxyethylene isosorbide (PI), polyoxyethylene isosorbide monooleate (PIM), polyoxyethylene isosorbide dioleate (PID), etc. Because the physicochemical behavior of polysorbate 80 as a surfactant is dependent upon its chemical composition, the variation of composition will affect the actual HLB of polysorbate 80. Therefore, quality analysis of polysorbate 80 from different sources is necessary for maintaining the performance and quality of the products. Direct MS analysis with ESI-MS or MALDI-TOF MS is not suitable for analyzing the complex sample because these methods firstly are not quantitative and secondly cannot differentiate some intermediates having the same molecular weights. An LC-MS method has been developed for quality analysis of polysorbate 80 in the presentation. The intermediates can be chromatographically separated and identified by MS. Furthermore, comparison of the normalized peak area of each component will indicate its quantitative difference of respective components in different polysorbate 80 materials.
Optimized UHPLC-MS Systems for Performance and Throughput: A Holistic Approach

UHPLC technology-driven markets are embedded most commonly in a regulatory environment that demands separation methodologies with speed, resolution, sensitivity, and numerous molecules to be discovered, identified and quantified in one chromatographic run. To meet these challenges on different markets analysts need UHPLC systems that provide seamless integration of all UHPLC modules while being hyphenated to mass spectrometers (MS). This prerequisite asks for systematic and profound examinations to identify the UHPLC-MS combinations with optimum holistic system performance. In this approach, we evaluate the impact of three different UHPLC systems on retention time (RT) reproducibility, peak properties, and sample throughput to ensure optimal MS performance and capacity utilization. We used binary high-pressure gradient (HPG) and quaternary low-pressure gradient (LPG) UHPLC systems with different pressure specifications, gradient slopes, columns at different flow rates, and we varied gradient delay volumes (GDVs) with different mixer volumes, leading to recommendations which type of analysis benefits most from the individual system configurations. UHPLC-MS analysis of pesticides has been used as a model representative for small molecule applications in this holistic approach.

Keywords: Chromatography, Food Safety, High Throughput Chemical Analysis, Liquid Chromatography/Mass Spec
Application Code: High-Throughput Chemical Analysis
Methodology Code: Liquid Chromatography/Mass Spectrometry
An LC-MS-TOF Method for Quantifying Components of Interest in Hemp Extract

Hemp extract contains high concentrations of cannabidiol (CBD) and low concentrations of Δ⁹-THC, the major psychoactive component of the cannabis plant from which CBD is extracted. Other compounds found in hemp oil include cannabinolic acid (CBNA), cannabinol (CBN), and cannabidiolic acid (CBDA). While liquid chromatography or GC-MS have traditionally been the method of choice for analysis of hemp extract, the utilization of an LC – MS - TOF allows for a single, more accurate method of quantifying all of these hemp extract components.

The method presented here allows for the quantitation of high concentrations of CBD and related components as well as the relative low concentrations of Δ⁹-THC within a single analysis. Analytical run times are 20 minutes. This run time is compensated for by shorter and more accurate sample preparation. Standards are diluted automatically with the use of an autosampler. Shorter run times (<10 min) may be achieved with more extensive sample preparation, but quantitation may be less accurate. The use of a custom Personal Compound Database and Library (PDCL) allows the analyst to verify identity data by matching accurate mass and retention times and avoid the errors that come with co-elution of analytes. Additional screening for toxic contaminants can be accomplished by comparison of the data to a PCDL.

Keywords: Flavor/Essential Oil, Liquid Chromatography/Mass Spectroscopy, Time of Flight MS

Application Code: Agriculture

Methodology Code: Liquid Chromatography/Mass Spectrometry
Two water-soluble vitamins with clinical research relevance are Vitamins B1 and B6. Thiamine Diphosphate (TDP) is the main biologically active form of Vitamin B1 and is required for various metabolic functions. Pyridoxal 5-phosphate (PLP) the main biologically active form of Vitamin B6 and is a coenzyme for a number of transamination reactions. Analysis of these micronutrients is important to diagnose potential deficiency which often occurs in elderly people due to malnutrition, bypass surgery and disease. Existing HPLC methods contain tedious derivatizations with long analysis time.

In this study, we develop a LC/MS/MS method including whole blood extraction procedure, internal standard (ISTD) to analyze both high polar TDP and PLP, using reverse phase analytical column without derivatization or ion paring reagent. While developing this method, real human blood matrix was examined and ways to move analyte peak away from matrix interference were explored in real sample matrix. We also evaluate 6 individual lots of human whole blood to average endogenous level of both TDP and PLP presented in healthy human, separation of the interference peaks and matrix effects were also monitored across all analytes. The validation was coupled with cost efficient and commercially available isotope ISTD, the results provide the assay dynamic range of 20 – 250 ng/mL with the linearity (R2) greater than 0.9939 for all compounds. The developed assay was accurate within 92.8-112%, and precise from 8.13-12.6% CV in human whole blood samples. The isotope internal standards response was consistent within ±25% of mean response of the standards and QCIs for all samples. Thus a simple, cost and time efficient method was developed and validated for the analysis of Vitamin B1 and B6 in human whole blood without the hassle of ion pairing or derivatizing agents.

**Keywords:** Clinical/Toxicology, Liquid Chromatography/Mass Spectroscopy, Tandem Mass Spec

**Application Code:** Clinical/Toxicology

**Methodology Code:** Liquid Chromatography/Mass Spectrometry
The increasing number of reports regarding food fraud scandals has brought food authenticity and safety to the attention of regulators, industry and consumers worldwide. Ambient ionization mass spectrometry (AMS) methods have overcome a number of intrinsic constraints of traditional mass spectrometric analysis schemes, allowing in situ, real-time analysis of a wide variety of samples. REIMS has been used for the analysis of human tissue during surgery and has shown to be capable of the identification of various tissue types based on lipid fingerprinting. In this study, we present an effective, near real time method to identify fish product speciation methods using REIMS.

REIMS was operated in single stage MS and negative mode. Data was acquired using a Medimass REIMS source coupled with a Waters Xevo G2-XS QTof mass spectrometer. All specimens were sampled using a monopolar handpiece that was equipped with a smoke evacuation line that was mounted on the atmospheric interface of the mass spectrometer. Full spectral information was recalibrated, normalised, baseline subtracted and binned up to 0.1 m/z bin size. The resulting data was subjected to multivariate analysis such as principal component analysis (PCA) followed by a linear discriminant analysis (LDA) using a non-commercial prototype software developed by Waters.

Over 3000 spectra were acquired from five different authenticated species of fish; cod, coley, haddock, pollock and whiting. Spectral data were acquired between m/z 200-1200. Both PCA and LDA score plots, built using m/z 600-950, identified clear signs of fish speciation. Validation of the PCA-LDA models carried out with another batch of fish samples resulted in a 94% correct classification rate.

REIMS technology could provide a paradigm shift across authenticity applications by providing real-time, reliable, and simple method for the analysis of food products.

Keywords: Food Identification, Food Safety, Mass Spectrometry, Time of Flight MS
Application Code: Food Safety
Methodology Code: Mass Spectrometry
One of the most challenging analytical separations for a scientist to attempt is the analysis of a polypeptide mixture. Depending on the sample, a scientist may be trying to separate polypeptides that may differ by subtle changes in the primary structure, glycosylation pattern, phosphorylation state, or any of a myriad of other physical or chemical differences. Due to this challenge, a number of different stationary phases have been developed, in recent years, as well as analytical methodologies to elicit separations of polypeptides. This talk will focus on employing different stationary phases and mobile phase modifiers to improve the selectivity of chromatographic methods involving polypeptides. Several mobile phase modifiers, including trifluoroacetic acid, difluoroacetic acid, formic acid, and ammonium formate, and their effects on the selectivity of peptides in a complex mixture, such as a tryptic digest, will be examined. In addition, an explanation for the effect of different stationary phase functionalities on the selectivity of various peptides in a complex mixture will be provided. Finally, aspects of method development involving the selection of appropriate stationary phase and mobile phase modifier will be discussed, using a series of insulin variants and analogs, whose primary structures only differ by one or two amino acids, as a case study.
Coccidiosis, a disease caused by the protozoa coccidia, commonly affects young cattle (2 weeks – 1 yr) that are confined to feedlots. Ionophore antimicrobials are administered to combat coccidiosis in young cattle and are also utilized to increase feed efficiency in adult dairy cattle. Only two ionophore antimicrobials are approved in the US for use in dairy cattle, monensin and lasalocid. However other ionophore antimicrobials are used in other countries. An analytical method utilizing solid phase extraction (SPE) followed by quantitation using liquid chromatography-tandem mass spectrometry (LC-MS/MS) has been developed to study six ionophore antimicrobials in both solid and liquid. Recoveries were found to range from 79-131%. Ionophore analysis was performed on solid and liquid manure samples taken from a dairy farm in Western New York, where a novel manure treatment system, Livestock Water Recycling (LWR), was installed. The efficacy of the LWR system to remove ionophore antimicrobials in liquid manure along various locations was assessed. During treatment, significant removal of monensin and lasalocid were not observed until after reverse osmosis treatment where monensin existed at a concentration of 8.1 ng/g and lasalocid existed below the limit of quantification (LOQ). Sampling various time points, ranging from one month before LWR was installed to one year after the system was installed, reveal no trend in removal for the liquid or solid manure concentrations for monensin and lasalocid over time. Though LWR acts as an attractive method for manure processing, ionophore residues persist and could act as a source of contamination depending upon the use of the clean water effluent.
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**Abstract Text**

There are many organic pollutants that affect our health. It is important to degrade these pollutants to reduce their harmful effects on human health and the ecosystem. The goal of this research is to study the oxidizing efficiency of ClO2 and O3 for breaking down various toxic organic compounds. The techniques of infrared spectrometry with a 10-meter gas cell and gas chromatography coupled to mass spectrometry (GC-MS) were used to study the degradation process of 1-bromopropane, dimethyl trisulfide, 2,3-butanedione and cadaverine. The initial concentration of 1-bromopropane was reduced significantly by ClO2 along with the generation of chlorinated by-products which were identified by GC-MS analysis. The treatment of dimethyl trisulfide against ClO2 produced many by-products like alkyl halide, sulfur dioxide and ethane sulfonyl chloride and methane sulfonyl chloride. Ammonia was the major by-product in the reaction between cadaverine and ClO2. Degradation products of 2,3 butanedione were formic acid, chloromethane and methylene chloride. The degradation efficiencies of these compounds are dependent on the concentrations of ClO2 and O3. The rates of degradation and the types of byproducts formed were noticeably different with the use of ClO2 compared to O3 and these will be discussed with full details.

**Keywords:** Environmental Analysis, FTIR, Gas Chromatography/Mass Spectrometry, Volatile Organic Compounds

**Application Code:** Environmental

**Methodology Code:** Gas Chromatography/Mass Spectrometry
In living organisms, oxidative stress (OS) results from an imbalance between the detrimental effects of reactive oxygen species (ROS) and the bioavailable anti-oxidants that prevent or repair damage to the host cells. ROS are naturally generated during normal mitochondrial respiration and by oxidative burst during immune response. Many factors may influence OS, including genetics, diet, exercise, and allergies. Some studies suggest that OS levels are increased following exposure to environmental toxicants, including tobacco smoke. A non-enzymatic peroxidation product of arachidonic acid, 8-isoprostane, may be measured in urine samples for estimating OS. Because we needed a robust, high through-put method that could be easily automated for large population studies, using 96 well plates we developed a solid phase extraction (SPE) ultrahigh performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) method for measuring urinary 8-isoprostane with a 100% detection rate. Since both glucuronidated and free forms of 8-isoprostane are present in urine, we enzymatically hydrolyzed the samples using glucuronidase prior to UPLC-MS/MS quantification. The ratio of conjugated and free forms varies among individual urines by 10% to 70%, therefore the measurement of the “total” (free and glucuronidated forms combined) gives a better estimate of OS levels than measuring the free form alone. Using 800 μL sample volume, this method returned a limit-of-detection (LOD) below 8 pg/mL and a between run coefficient of variation (CV) below 10% with a cycle time of less than 10 min.
HIV is a membrane-enveloped virus which causes acquired immunodeficiency syndrome (AIDS). An initial step of HIV infection is virus-host cell membrane fusion catalyzed by the viral fusion protein gp41. HFP is the ~25-residue N-terminal domain of gp41 and is required for membrane fusion with significant decreases in fusion activity with point mutations. The structures and membrane location of HFP are not yet well-understood nor is the HFP fusion mechanism. We investigated these by HFP 13CO to lipid/Chol 2H REDOR solid-state NMR. Our studies supported beta sheet conformation of HFP in membrane. Tight van der Waals contact between HFP and lipid/Chol was evidenced by ~4 Å 13CO-2H distances. In membrane without Chol, our studies have shown both deep and shallower membrane locations of HFP. There is also significant interest in Chol contact because both viral and host-cell membrane contains typically ~30mol% Chol. Our studies show the first atomic evidence of preferential contact between HFP and Chol over lipid supported by ~2 folds greater preference of HFP contacting with the isopropyl 2H nuclei of Chol than with the terminal acyl chain 2H nuclei of the lipid. At least in the absence of HFP, both these lipid and Chol 2H nuclei are located near the membrane center. Our studies support a role for Chol in membrane fusion and also demonstrate a new solid-state NMR capability for detection of specific affinity between membrane proteins and membrane lipid/Chol.

Keywords: Bioanalytical, Magnetic Resonance, NMR, Spectroscopy
Application Code: Bioanalytical
Methodology Code: Magnetic Resonance
Infection by influenza virus begins with endocytosis of the virus. The pH is reduced during endosome maturation and triggers a conformational change in the influenza hemagglutinin protein embedded in the viral membrane with subsequent fusion of the viral and ensosomal membranes. The ~25-residue N-terminal region of hemagglutinin is denoted the fusion peptide (IFP), binds to membrane, and is necessary for fusion. There have been several proposals for the IFP-induced changes in membrane organization which aid fusion catalysis. These include reduction in bending modulus, induction of negative curvature, stabilization of interstitial voids, and most recently, formation of lipid acyl chain hairpins with consequent increased exposure of lipid acyl chains to water. In the present study, such exposure was probed by measurement of lipid acyl chain 2H T2 relaxation rates in the absence and presence of IFP and the absence and presence of Mn2+. The lipids were 2H labeled either in the middle or tail regions of the acyl chains. For a liquid-crystalline membrane containing IFP, the Mn2+-relaxation rates increased by factors of 1.9 and 1.6 for the middle and tail 2H’s, which means a closer distance between lipid acyl chain and aqueous surface. Rotational-Echo, Double-Resonance NMR (REDOR) experiments also showed a close interaction between 13CO labeled peptide and 2H labeled lipid. Thus, IFP increases aqueous exposure of the hydrocarbon chains which supports acyl chain protrusion as one type of membrane perturbation induced by IFP.

Keywords: Bioanalytical, Magnetic Resonance, Peptides, Spectroscopy
Application Code: Bioanalytical
Methodology Code: Magnetic Resonance
Acute Kidney Injury (AKI) previously known as acute renal failure, refers to abrupt reduction in renal function or urine output, occurring over hours or days, caused by kidney damage due to sepsis, respiratory failure, heart failure, trauma, major surgery, burns, toxic insult caused by medications and contrast agents used for imaging. AKI accounts for 1% of all hospital admissions, complicates 7% of hospitalizations and is present in up to 20% of critically ill patients. Overall mortality associated with AKI is estimated at 45-70% mortality associated with AKI in intensive care unit patients requiring renal replacement therapy is 50-60% and more than 2 million die from AKI each year. The aim of the present study was to examine the effects of Ischemia induced AKI on the urine plasma and tissue metabolomic profile using NMR metabolomics tools. Ischemia reperfusion injury (IRI) was performed on swiss-webster mice, to induce AKI. Serum creatinine and urine neutrophil gelatinase-associated lipocalin levels were tested to confirm AKI after the surgery. Scanning electron and transmission electron microscopy images of the kidney tissue were captured to characterize the structural effects of the damage on the kidney tissue. Following NMR analysis of urine, plasma and tissue, twenty-two metabolites were identified as statistically significant as a result of damage.

Keywords: Metabolomics, Metabonomics, Microscopy, Plasma
Application Code: Biomedical
Methodology Code: Magnetic Resonance
The surface chemistry of nanoparticles strongly influences their resulting chemical and physical properties, and therefore also significantly influences the utility of these materials in a wide range of applications. A first step in both understanding and using nanoparticle surface chemistry is developing and implementing analytical methods to describe the chemical architectures present at the nanoparticle surface. Here, we compare the ligand exchange behaviors of silver nanoparticles synthesized in the presence of two different surface capping agents: poly(vinylpyrrolidone) (MW = 10 kDa or 40 kDa) or trisodium citrate, and under either ambient or low-oxygen conditions. In all cases, we find that the polymer capping agent exhibits features of a weakly bound ligand, producing better ligand exchange efficiencies with an incoming thiolated ligand compared to citrate and generating nanoparticles that are more susceptible to reactions with oxygen both during synthesis and ligand exchange. The influence of the original ligand on the outcome of ligand exchange reactions with an incoming thiolated ligand highlight important aspects of silver nanoparticle surface chemistry, crucial for applications ranging from photocatalysis to antimicrobials.

Keywords: Characterization, Material Science, Nanotechnology, NMR
Application Code: Material Science
Methodology Code: Magnetic Resonance
It is well-known that organic fluorine derivatives often show unusual properties and behavior in comparison with non-fluorinated parent compounds. Therefore, as part of our ongoing project on the synthesis of halogenated heterocyclic derivatives as possible bioactive compounds our research has been focused on exploring the synthesis of new fluorinated compounds.

In this particular case, we present the diastereoselective synthesis; the full characterization and the mechanism of formation of two novel trifluoromethyl derivatives of 2-(thio)oxoimidazolidin-4-one, made possible by a wise use of different spectroscopic techniques. A bicyclic that was determined as pyrrolo[1,2-c]imidazol-1-one and an intricate tricyclic, an pyrano[3',2':2,3]pyrrolo[1,2-c]imidazol-1-one. The reactions were performed in the absence of any solvent through a microwave assisted synthetic methodology. Both novel compounds were elucidated after a tricky analysis of their 1D and 2D NMR, CG-MS, IR, and UV spectra.

NMR spectroscopy was the major tool for the elucidation of the structure of both compounds. On the basis of one and two-dimensional NMR (1H, 13C, COSY, HSQC, HMBC and NOESY) we could settle their relative stereochemistry of reaction products.

Moreover, based on GC-MS and NMR data, it was possible to propose a mechanism for synthesis. UV-Vis spectroscopy and TD-DFT calculations allowed us to support the experimental data and therefore the proposed mechanism. Full details will be given at the poster session.

We would like to acknowledge the financial support by Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Fondo para la Investigación Científica y Tecnológica (FONCyT) and Secretaría de Ciencia y Tecnología (SeCyT - UNC). Lic. Faillace wants to thank CONICET for the fellowship granted.

**Keywords:** Drug Discovery, Gas Chromatography/Mass Spectrometry, Magnetic Resonance, UV-VIS Absorbance

**Application Code:** Drug Discovery

**Methodology Code:** Magnetic Resonance
To address the need for fast quality control of Brazilian gasoline fuel, this study introduces a compact 1H NMR spectrometer for the development of different chemometric models for rapidly determination and classification of several quality parameters of Brazilian gasolines such as specific gravity, flash point, benzene content, ethanol content, saturated, olefins and aromatics compounds content, and distillation temperatures to 10%, 50% and 90% of recovery. For all these studied parameters, the developed models showed margins of error better or comparable than reference analytical techniques proposed and applied by the Brazilian National Agency of Petroleum (ANP). Moreover, the gasoline analysis by the compact low-field NMR spectrometer is simple and fast. It needs no more than 30 seconds, because no sample pre-treatment or dilution in deuterated solvents is required and the NMR spectra is acquired by single-scan analysis. This is a tremendous gain in analytical frequency when compared to the reference methodologies proposed by the ANP, which most of them are based on physical-chemical tests that take hours to be performed and also need specialized technicians.

Keywords: Fuels\Energy\Petrochemical, NMR, Process Analytical Chemistry, Quality
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Magnetic Resonance
In this study we demonstrate the high sensitivity of our in-house built electrokinetic sheath flow interface to couple capillary zone electrophoresis to a Thermo QE-HF mass spectrometer (CZE-ESI-MS). This interface is designed to provide exquisite sensitivity with a robust performance. HF-etched capillaries employed in these studies add to the sensitivity of the interface, while narrow inner diameters increase the resolution of the resulting electropherograms.

In these studies, we have examined this performance by determining a limit of detection via single reaction monitoring using a standard bovine serum albumin (BSA) tryptic digest spiked with an exact amounts of angiotensin II. A calibration curve of spiked angiotensin II in a constant concentration of the BSA digest showed linearity from 100 zeptomole to 10 femtomole loading amounts. Further dilutions of angiotensin II showed reproducible signals for 10 zeptomole loading amounts, and a limit of detection on the order of yoctomole loading amounts. Preliminary results of studies with even narrower ID capillaries have shown reproducible signal for 1 zeptomole loading amounts.

Further studies are ongoing in the detection of low concentration peptides naturally found within a protein digests of BSA and E. coli cells. In these experiments, we aim to build a library of accurate mass tags for a digest, and then apply this library to the analysis of small samples (i.e. single cells). The results of these studies will shed light on the system’s usefulness for applications in proteomics and metabolomics studies. Future potential systems of interest include late stage [i]Xenopus laevis[/i] blastomeres and possible enzyme studies.

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

Application Code: Genomics, Proteomics and Other ‘Omic

Methodology Code: Capillary Electrophoresis
In 2014, a naturally occurring cyanobacteria bloom in the Lake Erie resulted in high levels of microcystin (MC), a hepatotoxin that shut down the municipal water supply in Toledo. Events like this have increased the awareness of water contamination, and underscored the need for quick and easy methods for water quality monitoring. Current methods to detect MC in water are expensive, time consuming, and often require transportation of the sample to the laboratory. An aptamer-based in situ method could reduce water quality testing time and cost. Capillary electrophoresis (CE) has been used as an efficient separation method when selecting aptamers for proteins. However, MC is a much smaller molecule, which when bound to DNA sequences, results in little to no change in electrophoretic mobility and poor separation from the unbound sequences. To address this, we have conjugated MC to quantum dots (QD), which effectively makes the MC target larger, and allows efficient separation based on the QD’s mobility. Conjugation of the QD to MC was accomplished via a cysteamine linker between the seventh amino acid residue and the terminal carboxyl group on the QDs. The formation of the cysteamine-MC complex was confirmed by MALDI mass spectrometry. After incubation of the QD-MC complex with a random sequence DNA library, QD-MC-DNA complexes were separated from unbound DNA and collected using CE. Denaturing PAGE was used to regenerate single-stranded DNA from the PCR products for subsequent rounds of selection. Fluorescence polarization was used to determine dissociation constants. Results will be presented.

This research was supported by the Provost’s Research Support Award (JG), the Undergraduate Research Stimulus Program (MB,CR), the Women in Philanthropy Grant (MB) and the Department of Chemistry at Eastern Michigan University.

Keywords: Bioanalytical, Biosensors, Capillary Electrophoresis, Method Development

Application Code: Bioanalytical

Methodology Code: Capillary Electrophoresis
Sensitive and Fast Characterization of Site-Specific Protein Glycosylation with Capillary Electrophoresis-Electrospray Ionization-Mass Spectrometry

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Guijie Zhu, Liangliang Sun, Norman J. Dovichi, Zhenbin Zhang

Abstract Text

Site-specific characterization of glycopeptides is a prerequisite for fully understanding the biochemical and physiological functions of glycoproteins. Aberrant glycan microheterogeneity has been revealed to implicate in a variety of disease, such as cancer. The great diagnostic potential in biomedical field necessitates the study on qualitative and quantitative characterization of site-specific glycan heterogeneity. However, the overall complexity as well as the limited amounts of available biological matrixes often brings the difficulty in Mass spectrometry analysis. Therefore, the development of efficient separation and high sensitive detection methodology for glycopeptide characterization is indispensable.

Capillary-zone electrophoresis (CZE) is well known to allow the high-resolution and high-sensitivity analysis of wide range of biological molecules after interfaced with mass spectrometry (MS). Our group developed an electrokinetically pumped sheath-flow nanospray interface, which dramatically have the detection stability and sensitivity dramatically improved for CZE-ESI-MS analysis.

In this work, we extend the application of our sheath-flow interface assisted CZE-ESI-MS platform to the analysis of protein glycosylation. By analyzing the model glycoprotein, successful separation of glycopeptides within as less as ~5 min was achieved, especially with the observation of differentially terminal-galactosylated and sialylated species based on the same glycosylation sites being well resolved. Two order of magnitude improvements in detection limit was obtained, in comparision with convention nanoLC-MS method. We also observed a 1.5% and 7.1% average relative standard deviation in peak migration time and glycopeptide relative abundance. All the results demonstrated the great promise of our CZE-MS/MS setup in fast and sensitive analysis of site-specific glycan heterogeneity.

Keywords: Capillary Electrophoresis, Mass Spectrometry, Protein
Application Code: Genomics, Proteomics and Other 'Omics
Methodology Code: Capillary Electrophoresis
The past few years have seen dramatic improvements in the speed of chromatographic enantioseparations. Nevertheless, routine chromatographic determinations of enantiopurity to support stereochemical investigations in pharmaceutical research and development, synthetic chemistry and bioanalysis are still typically performed on the 5-20 min timescale, with many practitioners believing that sub-minute enantioseparations are not representative of the molecules encountered in day to day research. In this study we develop ultrafast chromatographic enantioseparations for a variety of pharmaceutically-related drugs and intermediates, showing that sub-minute resolutions are now possible in the vast majority of cases. An impressive collection of “world speed records” for chromatographic enantioseparations is also presented.
To differentiate MabSelect SuRe sepharose from other resins, especially very similar protein-based sepharose subtypes, a solvent-free Near Infrared (NIR) and visible spectroscopic method has been developed and validated.

To build the spectral library system, 10-30 reference spectra for each resin analyte were gathered. Method development involved optimizing library configuration and parameters, and validating the identification method. Cascading library configurations, a first tier global identification and two more tiers for local qualifications, were optimized. Various spectral pretreatments and chemometric methods were investigated to maximize identification ability in each tier of the library system. Wavelength correlation was exploited to a narrow visible spectral section where the curves of MabSelect SuRe and MabSelect sepharose move in opposite directions. During method validation, negative challenge resins, positive control samples and long-term variation were examined.

A global identification (1st tier library) used three wavelength segments and spectral pretreatments (SNV and Savitzky-Golay Second Derivative) to exclude moisture bands and to minimize the impact from sample preparation and environmental variations. Ten of thirteen resin analytes reached clear identifications. Ambiguous MabSelect SuRe, rProtein A and MabSelect sepharoses were then searched by 2nd tier local libraries. MabSelect SuRe interference to MabSelect sepharose was excluded and rProtein A and MabSelect sepharoses got identified. In the 3rd tier library, a tiny visible region was selected to achieve MabSelect SuRe sepharose final identification. 38 additional negative challenge resins were successfully examined. Long-term variability had no adverse effect on identification of MabSelect SuRe sepharose.

This NIR and visible spectroscopic method with a reliable three-tier library system is selective and fast to identify MabSelect SuRe sepharose.

Keywords: Identification, Infrared and Raman, Method Development, Pharmaceutical
Application Code: Pharmaceutical
Methodology Code: Molecular Spectroscopy
Pharmaceuticals

Hydrophilic Interaction Liquid Chromatographic Determination of Glimepiride in Pharmaceutical Formulations

Glimepiride is one of the most widely prescribed antidiabetic drugs and contains both hydrophobic and hydrophilic functional groups in its molecules, and thus could be analyzed by either reversed-phase (RP) or hydrophilic interaction liquid chromatography. In the literature, however, only RP high performance liquid chromatography (HPLC) has been reported. In this study, a simple, rapid and accurate hydrophilic interaction liquid chromatographic (HILIC) method was developed for the determination of glimepiride in pharmaceutical formulations. The analytical method comprised a fast ultrasound-assisted extraction with acetonitrile as a solvent followed by HILIC separation and quantification using a Waters Spherisorb S5NH2 hydrophilic column with a mobile phase consisting of acetonitrile and aqueous acetate buffer (5.0 mM). The retention time of glimepiride increased slightly with decrease of mobile phase pH value and of acetonitrile content, indicating that both hydrophilic, ionic, and hydrophobic interactions were involved in the HILIC retention and elution mechanisms. Quantitation was carried out by relating the peak area of glimepiride to that of the internal standard, with a detection limit of 15.0 µg/L. UV light absorption responses at 228 nm were linear over a wide concentration range from 0.00 µg/L to 6.00 mg/L. The recoveries of the standard added to pharmaceutical tablet samples were 99.4 – 103.0% for glimepiride, and the relative standard deviation for the analyte was less than 1.0%. This method has been successfully applied to determine the glimepiride contents in pharmaceutical formulations.

Abstract Text

Glimepiride is one of the most widely prescribed antidiabetic drugs and contains both hydrophobic and hydrophilic functional groups in its molecules, and thus could be analyzed by either reversed-phase (RP) or hydrophilic interaction liquid chromatography. In the literature, however, only RP high performance liquid chromatography (HPLC) has been reported. In this study, a simple, rapid and accurate hydrophilic interaction liquid chromatographic (HILIC) method was developed for the determination of glimepiride in pharmaceutical formulations. The analytical method comprised a fast ultrasound-assisted extraction with acetonitrile as a solvent followed by HILIC separation and quantification using a Waters Spherisorb S5NH2 hydrophilic column with a mobile phase consisting of acetonitrile and aqueous acetate buffer (5.0 mM). The retention time of glimepiride increased slightly with decrease of mobile phase pH value and of acetonitrile content, indicating that both hydrophilic, ionic, and hydrophobic interactions were involved in the HILIC retention and elution mechanisms. Quantitation was carried out by relating the peak area of glimepiride to that of the internal standard, with a detection limit of 15.0 µg/L. UV light absorption responses at 228 nm were linear over a wide concentration range from 0.00 µg/L to 6.00 mg/L. The recoveries of the standard added to pharmaceutical tablet samples were 99.4 – 103.0% for glimepiride, and the relative standard deviation for the analyte was less than 1.0%. This method has been successfully applied to determine the glimepiride contents in pharmaceutical formulations.

Keywords: Chromatography, HPLC, Pharmaceutical, Quantitative
Application Code: Pharmaceutical
Methodology Code: Liquid Chromatography
Optimization of an Innovative Sampling Method for Air Sampling of Semi Volatile Organic Compounds

This work describes optimisation steps of an innovative method for the measurement of several groups of semi-volatile organic compounds (SVOCs) in air, collecting both gaseous and particulate air fractions. The method is based on low volume active air sampling on mixed-bed sorption tubes (consisting of polydimethylsiloxane (PDMS) and Tenax TA), followed by thermal desorption and gas chromatography mass spectrometry analysis (TD-GC-MS). The optimised method was validated for the measurement of selected target compounds from the following chemical classes: polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), organophosphate (PFRs) and “novel” brominated (NBFRs) flame retardants, and phthalate esters (PEs). The method is characterised by limits of detection in the range of 0.003–0.3 ng m\(^{-3}\) for PAHs, 0.004–0.2 ng m\(^{-3}\) for PCBs, 0.006–0.025 ng m\(^{-3}\) for PBDEs, 0.006–0.171 ng m\(^{-3}\) for PFRs, and 0.007–0.041 ng m\(^{-3}\) for NBFRs and 0.002–0.2 ng m\(^{-3}\) for PEs, recoveries >88%, a linearity of 0.996 and a repeatability of <10% for all studied compounds.

The method was applied in different Belgian urban outdoor as well as indoor environments. Additionally, it was also compared with a standard method for measurement of PBDEs in air using active air sampling on XAD-2 sorbent material, followed by liquid extraction. This air sampling method showed a good efficiency for the determination of selected members of different SVOC classes in one sorption tube. The use of low flow air sampling equipment makes this technique advantageous for applications in indoor environments or for personal sampling as well.

Keywords: Environmental/Air, PAH, Sample Preparation, Thermal Desorption
Application Code: Environmental
Methodology Code: Sampling and Sample Preparation
Polycyclic aromatic hydrocarbons (PAHs) are widely recognized as a particularly harmful group of organic compounds. Resulting from the incomplete combustion of coal, gasoline and other organic materials, they are particularly prevalent in urban and industrial environments, which combined with their toxicity, has resulted in very low limit levels for urban and workplace air. This means that the lower-volatility PAHs tend to occur bound to particulate matter, as well as being present in the air, and this is reflected in global standardized methods for the analysis of PAHs. However, the analysis of these compounds requires labor-intensive liquid extraction, which makes these methods difficult to automate. Consequently, there is demand for more efficient techniques for the sampling of PAHs and their introduction to the GC system. 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. This is complemented by the ability of modern thermal desorbers to automatically add internal standards and the option of re-analysis of the same sample using the splitting and re-collection capability.

In this poster, we describe the development and validation of a TD–GC–MS method for the analysis of PAHs, using a new generation of thermal desorbers with outstanding capabilities for these challenging analytes.

**Keywords:** PAH, Sample Handling/Automation, Sample Preparation, Thermal Desorption

**Application Code:** Validation

**Methodology Code:** Sampling and Sample Preparation
Water quality is of the utmost importance and recently the importance of analyzing water for emerging contaminants has been brought to light. Among the emerging compounds being analyzed are perfluorinated chemicals (PFCs) which have been found to be persistent environmental contaminants derived from various industries. For example, perfluorooctane sulfonate (PFOS) has been used in a number of different industries, including the semiconductor and photographic industries, in some firefighting foams and in hydraulic fluids used in the aviation industry. Modern analytical labs are looking to automation to help reduce solvent usage and increase sample throughput while ensuring the high quality of the resulting data.

A single X-Y-Z coordinate autosampler commonly used for sample introduction in GC or HPLC can be used to perform a wide variety of sample preparation techniques using a single instrument and controlling software. The sampler can be configured as part of the LC/MS/MS system.

In this report, the complete automation of an on-line SPE-LC/MS/MS method used for the determination of perfluorinated compounds in water samples is discussed. Calibration curves were prepared and limits of quantitation were determined at levels near 0.01 ng/mL for all tested PFCs using a 1mL sample volume. The average precision for the PFCs examined ranged from 1.73% to 11.7% CV and the average accuracy for the PFCs examined ranged from 90.6% to 110%.

Keywords: Environmental/Water, Liquid Chromatography/Mass Spectroscopy, Sample Preparation, Solid Phase
Application Code: Environmental
Methodology Code: Sampling and Sample Preparation
Sampling and Sample Preparation: MS, SPE, and SPME

Dispersive Solid-Phase Extraction and In-Vial Filtration as a Simplified Clean-Up for Ethylphenols Determination in Red Wines

Brettanomyces sp. is a microorganism related to several wine faults, most notably those known as phenolic off-flavor or Brett character. This flavor can be described as horsey, leathery, medicinal, smoky or savory being caused by the presence of ethylphenols (EPs). Although Brettanomyces is considered a spoilage organism that cause an objectionable flavor in red wine when their related spoilage compounds are present in high levels, low levels of EPs are sometimes considered adding complexity to a wine. Due to the relevant organoleptic impact of EPs on wine quality at variable levels, the development of analytical methodologies for the determination of them in wine samples is a subject of interest.

In this work, we report the development and validation of a new high-throughput analytical method to monitor EPs in red wines using a quick, easy, cheap, effective, rugged and safe (QuEChERS) sample preparation followed by HPLC-UV determination. The approach combines in-vial filtration with dispersive solid-phase extraction (d-SPE) in a fast and convenient clean-up of QuEChERS extracts. During method development, different commercial sorbents for d-SPE were investigated and compared with respect to analyte recoveries. The method was validated at 0.1, 0.5 and 5 mg L⁻¹ spiking levels, and the results for all analytes recoveries were between 72 and 119 % with RSD □ 15 %. The developed method was applied to several samples; being 4-ethylphenol and 4-vinylguaiacol the compounds detected with major frequency. The presented approach has high sample throughput and usefulness in screening studies for wines Brett character analysis.

Keywords: Beverage, Extraction, Food Science, High Throughput Chemical Analysis
Application Code: High-Throughput Chemical Analysis
Methodology Code: Sampling and Sample Preparation
The research presented a new approach for diffusive sampling of airborne polar compounds (PC) based on solid-phase microextraction (SPME) for occupational exposure assessment. It is focused on automation and miniaturization for multiple and specific fast gas chromatography (GC) analysis by flame ionization, electron capture and mass spectrometry detectors. O-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine hydrochloride, 1-pirenyldiazomethane, hydrogen bromide, methyl-p-tolyl-sulfide, triphenylphosphine derivatization reagents for on-fiber sampling of aldehydes, fatty acids, ethylene and propylene oxide, peracetic acid and hydrogen peroxide were studied. Important parameters influencing the extraction and derivatization process such as type of fiber coating, type and volume of derivatizing reagent, extraction time, and desorption conditions were investigated and optimized. The automation of the preparation and injection procedure by new device, through change of the SPME fibers, allowed a friendly use of fast GC apparatus with a number of advantages including reduced analyst time and greater reproducibility (2.31-5.79%). PC vapours were generated by use of a syringe pump in a dynamic system in which temperature, relative humidity, and air velocity were monitored. The theoretical sampling rate for time-weighted average and rapid- and TWA-SPME were, furthermore, estimated by use of the Fuller-Schettler-Giddings diffusion coefficient and were in accordance with experimental values. The detection limits were less than 0.030 ug/mc. The compared results obtained using current and standard methods were shown to be satisfactory. So, the introduction of dedicated, automated, and robotic systems allowed a friendly use of fast GC apparatus for high-throughput screening so as to reduce the costs of the monitoring campaigns.

Keywords: Clinical/Toxicology, Environmental/Air, SPME
Application Code: Clinical/Toxicology
Methodology Code: Sampling and Sample Preparation
Solid phase micro-extraction is widely used as a valid solvent-free pre-concentration technique in the analysis of a wide range of pollutants in environmental water samples. However, since its introduction, the technique remained almost unchanged, retaining its limitations in terms of fiber phase volume and mechanical robustness. Mechanical robustness is a key critical challenge to widely adopt automatically this technology.

This paper describes an advancement made integrating the fiber within the syringe needle that allows combining the advantages of the standard SPME together with a larger fiber phase volume, and an increased sample throughput and robustness.

In this study, an evaluation of this new SPME technology is presented for the determination of the 16 EPA regulated Polycyclic Aromatic Hydrocarbons in water. Parameters optimized for Direct Immersion SPME along with results on sensitivity, repeatability and linearity obtained with a GC-Single Quad MS system are shown.

Data demonstrates the enhanced capability of this system to reach detection limits down to pg L−1 level and the improved mechanical reliability, suggesting this new technology as a valid alternative method for the automated and quantitative analysis of PAHs in water as well as for a wide variety of other potential applications.
Sampling and Sample Preparation: MS, SPE, and SPME

Application of High Efficient Concentrator for Sample Preparation

Concentration process is commonly applied to reduce the sample volume and improve the detection limit of analytes. Automatic multiple concentrator can efficiently improve sample preparation process. LabTech MV5 and M8 concentrators can simultaneously treat up to 54 and 8 samples, respectively. With the water bath heating and nitrogen vortex, the evaporation concentration is highly processed. The front window and inside lighting of the instruments make the evaporation process easily be observed without opening the bath cover and interrupting the evaporation process. In this poster, the evaporation efficiencies and analyte recovery were studied and reported.

Keywords: Agricultural, Environmental, Environmental Analysis, Food Science

Application Code: Environmental

Methodology Code: Sampling and Sample Preparation
EPA Method 525.2 is used to determine a wide array of organic compounds in raw and finished drinking waters. One (1) liter water samples are passed through chemically bonded C18 solid phase extraction (SPE) cartridges or disks. The analytes of interest are recovered with ethyl acetate and dichloromethane bottle washes which are used as elution solvent for analyte recovery from the dried sorbent bed. 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).

A fully-automated Large-Volume Injection (LVi) system allows the user to pass large volumes of aqueous sample matrix through commercially available SPE cartridges and disks. The autosampler will accommodate 1 liter sample jars, therefore sample is taken directly from the container used to collect the water in the field. The sample jar rinses are used to elute the analytes of interest to collection tubes, or to a 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 to the instrument and 2) programming the sequence with saved method parameters. This provided for a fast and simple automated method for the labor intensive process of manually loading 1 liter of water to an SPE, subsequent elution, and concentration for analysis. An evaluation of several commercially available C18 SPE cartridges and disks was performed.

Keywords: Pesticides, Sample Preparation, Solid Phase Extraction, Water
Application Code: Environmental
Methodology Code: Sampling and Sample Preparation
Sampling and Sample Preparation: MS, SPE, and SPME

Analysis of 25-Hydroxyvitamin D2/D3 in Serum by Cleanert SLE Plates Couple with LC-MS/MS

Background: The level of 25-hydroxyvitamin D3 (25-OH VD3) and D2 (25-OH VD2) in human body is an important factor for clinical application. Recently, LC-MS/MS has been accepted as a standard method for determination of 25-OH VD2/VD3 in plasma and serum. It is no doubt a clean-up procedure is necessary to remove the interferences from biosamples prior to LC-MS/MS.

Method: A simplified clean-up procedure utilizing supported liquid extraction (SLE) plates was developed. 10 μL of internal standard (hexadeuterated 25-hydroxyvitamin D3 and Trideuterated 25-hydroxyvitamin D2) was added to 100 μL serum and vortexed for 30s. The serum was diluted with 100 μL of 50mM NaOH. The mixture was loaded onto Cleanert SLE plate and wait for 5 min for fully adsorption of the aqueous phase on the solid phase of the plate. The target compounds were eluted with 4 x 500 μL of isooctane with an interval of 5 min and collected into a deep well plate. Cleanert M96 positive pressure device was used remove the residue of organic phase from Cleanert SLE plate. The fractions in collection plate were evaporated to dryness and reconstituted with 200 μL of methanol / water (80/20,v/v) containing 0.1% of formic acid. The treated samples were detected by LC-MS/MS coupled with a Durashell C18-L HPLC column. Quantification of 25-OH VD3 and 25-OH VD2 were conducted with electrospray ionization MS/MS in the multiple-reaction monitoring mode.

Results: The lower limit of quantitation was 2 ng/mL for 25-OH VD3 and 4 ng/mL for 25-OH VD2. The calibration was linear up to 500ng/mL for 25-OH VD3 (r2=0.995) and 1000ng/mL for 25-OH VD2 (r2=0.999). The recoveries of 25-OH VD3 in a range from 87.2% to 104%, while 25-OH VD2 from 85.4% to 111% respectively. No ion suppression or enhancement was observed. The matrix effect factor was less 10%. Comparing with protein precipitation couple with liquid-liquid extraction, the operation steps of SLE was much less and more clean samples were obtained.

Keywords: Bioanalytical, Clinical/Toxicology, Liquid Chromatography/Mass Spectroscopy, Sample Preparation
Application Code: Clinical/Toxicology
Methodology Code: Sampling and Sample Preparation
EPA Method 3640A for Gel-Permeation Chromatography (GPC) cleanup uses 70g of SX3 styrene-divinylbenzene Biobeads and requires 65 minutes of run time using DCM as a mobile phase. The maximum lipid loading capacity for this traditional column is 1 gram. With the introduction of the smaller-scale column, run times were shortened to 40 minutes with a corresponding reduction in solvent usage. However, the maximum lipid loading for the column was also reduced to 500mg. A propriety stationary phase has been developed that reduces the run time for both the 1-gram and 500mg lipid-loading capacity columns while maintaining the EPA method's resolution and analyte recovery requirements. Comparisons between the traditional EPA column, the small-scale column and the new columns will be presented.
Organochlorine-pesticides (OCPs), polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) are important, historical contaminants which are routinely monitored in environmental samples. These lipophilic compound classes are routinely found in biological tissues from aquatic environments owing to their persistence for accumulating in lipid, fats and oils, increased concentration up the food chain, and their low propensity for degradation in the environment. While many of these chemicals have been banned from manufacture and use in the United States since the late 1970s to the early 1980s, these characteristics of persistence and accumulation make them important priority organic pollutants to monitor in aquatic tissues for consumption advisories, endocrine disruption effects and general species health.

Due to the historic nature of these compound classes, methods for their extraction, isolation from co-extracted materials, and instrumental quantification are well established. However, most methods are time consuming and require manual handling at each step of the sample preparation process. Using a fully automated system, lipid-rich aquatic tissue raw extracts were processed through automated, programmable methods to a final sample ready for gas chromatography-electron capture detection (GC/ECD) quantification. Gel permeation chromatography (GPC) followed by solid phase extraction (SPE) clean-up and fractionation resulted in two fractions for GC/ECD analysis with negligible manual manipulations. Fraction 1 (F1) contained PCBs and a few non-polar OCPs, and Fraction 2 (F2) contained the more polar OCPs and PBDEs.
**Session Title**: Sampling and Sample Preparation: MS, SPE, and SPME  
**Abstract Title**: Analysis of 25-Hydroxyvitamin D2/D3 in Whole Blood Microsampling by Cleanert PEP MicroPlates Couple with LC-MS/MS

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### Abstract Text

**Background**: In the last decade, it has been realized that low levels of vitamin D is associated with a range of adverse health outcomes. Currently, most clinical methods available for vitamin D quantitation require large sample volume, which is cumbersome for venous blood sampling in newborns. Therefore, a method of vitamin D measurement compatible with microsampling is needed for neonates. The objective of this study was to develop an LC-MS/MS method for the measurement of 25OH vitamin D2 and 25OH vitamin D3 in neonates using three whole blood microsampling methods (whole blood, dry blood spot (DBS) and the MitraTM Microsampler (Neoteryx, CA, USA)) (20 l whole blood used in all the three methods).

**Method**: DBS and MitraTM Microsampler were extracted under ultrasonic by 50 l 0.2M ZnSO4 and 200 l methanol with internal substance, while protein precipitation whole blood by 50 l 0.2M ZnSO4 and 200 l methanol with internal substance. Centrifuge the sample at 10000 rpm for 5 min, the supernatant were loaded onto Cleanert PEP MicroPlate which was pre-conditioned by methanol and 60% methanol aqueous solution. The plate was washed with 5% methanol and 60% methanol, then discard the elute solution. Target compounds were eluted by 80 l methanol/IPA (95/5, v/v), 30 l water was added into d elute solution, votex for 30s. Analytes were separated by Kinetex XB-C18 LC column and detected by SCIEX Triple Quad5500 mass spectrometer.

**Result**: The calibration curve was constructed using the artificial vitamin D-free blood and six-level calibrators (2, 5, 10, 25, 50, 100 ng/ml for D2 and 4, 10, 20, 50, 100, 200 ng/ml for D3. In method validation, the developed assay showed analytically acceptable performance in linearity and within-run precision). The result shown Cleanert PEP MicroPlate was suitable for high throughput measurement of 25OH vitamin D2 and 25OH vitamin D3 in neonates using 20 lbf whole blood.

**Keywords**: Clinical Chemistry, Liquid Chromatography/Mass Spectroscopy, Sample Preparation, Solid Phase Extr

**Application Code**: Clinical/Toxicology

**Methodology Code**: Sampling and Sample Preparation
The use of magnetic ionic liquids (MILs) for extractions and other applications is a rapidly growing field in analytical chemistry. The careful design and synthesis of these MILs for task specific analytical challenges make them both novel and exciting. However, designing the perfect MIL is no small feat and many MILs that have excelled in one or two desirable properties have also met adversity in other aspects. Hydrophobicity, viscosity, room temperature liquids, high magnetic susceptibility, selectivity, and thermal stability are all desirable properties when using MILs for various downstream applications. In this study, the hexafluoroacetylacetone ligand has been coordinated to transition and rare earth metals to create a highly hydrophobic anion. Paired with the highly hydrophobic P66614+ cation, room temperature MILs with relatively low viscosity and high hydrophobicity have been created. Furthermore, high magnetic susceptibility has been achieved by the addition of gadolinium and dysprosium based anions. There are relatively few room temperature rare earth MILs that have been reported, but the rare earth repertoire has been expanded by this synthesis. These MILs can be synthesized in two steps; a one pot synthesis of the anion followed by a metathesis reaction of the anion with trihexyltetradecylphosphonium chloride. This adds to the simplicity and practicality of using these MILs for downstream applications.

Keywords: Isolation/Purification, Sample Preparation, Separation Sciences
Application Code: General Interest
Methodology Code: Separation Sciences
The detection of pathogenic bacteria within liquid samples is vital within the fields of food chemistry, environmental microbiology, and clinical research. Conventional approaches for isolating and identifying bacteria rely on culture-based methods that often require a time-consuming process before concluding the absence or presence of the cells of interest. In this study, two hydrophobic magnetic ionic liquids (MILs), both containing the trihexyl(tetradecyl)phosphonium ([P66614+]) cation with the anion component consisting of hexafluoroacetylacetonate ligands coordinating to either a nickel(II) or cobalt(II) center (Ni[F6acac]₃, Co[F6acac]₃) were investigated for the rapid extraction and analysis of bacterial cells from aqueous solution. Following a rapid dispersive extraction, the cell-enriched MIL microdroplets were isolated from an aqueous solution by applying an external magnetic field. The bacterial cells were recovered from the MIL extraction solvent using luria-broth (LB) and subsequently analyzed by two methods: (1) PCR amplification to detect a DNA sequence characteristic of the microorganism and (2) agar plate counting to verify the presence of live bacteria in the sample. A target gene was successfully amplified following a 30 s dispersive extraction from an aqueous solution containing approximately 17,000 cells mL⁻¹. Additionally, quantification of the number of cells extracted by method (2) revealed a 16-fold increase in colony forming units per milliliter (CFU mL⁻¹) when compared to the aqueous sample. The optimized extraction method using MILs as extraction solvents for bacterial cells highlights a promising avenue for the rapid identification of cells within liquid samples.

**Keywords:** Bioanalytical, Environmental/Biological Samples, Food Safety, Sample Preparation

**Application Code:** General Interest

**Methodology Code:** Sampling and Sample Preparation
Separation Science

Modification of Core-Shell Silica Particles with a Carbonaceous Layer for HPLC

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 providing 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 layer on silica particles via diazonium chemistry consisting of numerous interconnected aromatic rings with amine functionalities. Subsequently, the modified silica was heated under an inert atmosphere of argon gas, resulting in a carbonaceous layer at the silica surface. The modified silicas, prior and after heating, were characterized by diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS), thermogravimetric analysis, and elemental analysis; these confirmed the proposed structure of the chemical layers on the silica surface. Nitrogen gas adsorption measurements (e.g., Brunauer-Emmit-Teller or BET analysis) of the synthesized materials provided insight into the pore structure of the modified silica. The unheated modified silica contains amine groups that can act as adsorptive sites in LC and also provide a means to further functionalization if desired. Columns have been packed with both materials (prior and after heating) and preliminary chromatographic testing has been initiated. This presentation will focus on the details of the synthesis, characterization, and the preliminary chromatographic testing of the carbonaceous material for LC.

Keywords: HPLC, HPLC Columns
Application Code: Other
Methodology Code: Liquid Chromatography
Asymmetric flow field flow fractionation (AF4) is an efficient size based separations technic for the characterization of submicron size particulates. In AF4, membranes having various molecular weights cut off sizes are used as barrier to retain particles while allowing carrier fluid containing electrolytes to permeate. Here we hypothesized the possibility of electrolyte rejection by barrier membrane and their accumulation in the channel during operation. The electrolyte accumulation can cause various adverse effects on analytes including membrane fouling. An instrument setup containing conductivity detector was assembled and rejection of commonly used carrier electrolytes such as tri sodium citrate, ethalinediaminetetraacetic acid, sodium chloride and ammonium carbonate were evaluated by varying concentration, cross flow rate, focusing flow rate, membrane material type, and cutoff sizes. The results showed that electrolyte rejection was increased with decrease of electrolyte concentration and molecular weight cut of size (pore size) and increase of charge state of anion in the carrier electrolytes. We proposed an electrostatic repulsion based rejection mechanism and verified it with measurement of rejection rate varying electrolyte concentration in the running media.
Field-Flow fractionation (FFF) is an elution-based separation technique which is capable of the rapid and high efficient separation of macromolecules, colloids and particles. Asymmetrical flow FFF (AF4) is one of the FFF sub-techniques that has been broadly used in characterization of complex biological and pharmaceutical products. The commercial AF4 system commonly employs flat channels with different dimensions and aspect ratios (10-28 cm long and 2-10 cm wide). Annular channels (hollow fiber) have been also used in the AF4 system as an alternative geometry. The AF4 channel could be further downscaled to reduce channel volume (less dilution), sample consumption and operating cost. A miniaturized flat AF4 channel (microchannel) with a size smaller than a credit card was constructed and examined for characterization of biological samples spanning a wide molecular weight and diameter range. The performance of the microchannel was tested using a mixture of plasma proteins. The resolution and reproducibility of the microchannel were found to be similar to those of standard channel. The microchannel was used to fractionate Human Serum Albumin (HAS) from different lipoprotein fractions. The baseline separation was also achieved between the high-density and low-density lipoproteins. The efficiency of the microchannel was examined to separate linear DNA fragments. The results demonstrated the separation of different DNA base pairs linear fragments.

Keywords:  Bioanalytical, Environmental/Biological Samples, Method Development, Separation Sciences

Application Code:  Bioanalytical

Methodology Code:  Separation Sciences
Heavy metal exposure is associated with increased risk for developing breast cancer. We have recently extended these findings in an epidemiological study that reported increased levels of urinary lead and copper in women with untreated breast cancer. However, the availability of analytical methods for comprehensive analysis of the urine metallome is limited by trace levels of some metals in urine. In this study, we developed novel adsorbents for urinary metal preconcentration using modified rice hull adsorbents. Treated lignocellulose, the primary component of rice hull, possessed surface functionalities that include hydroxyl, carboxyl, and phenolic groups that exhibited electrostatic interactions with monovalent and divalent metal ions. Adsorption and desorption kinetics were evaluated with respect to solution pH, composition, contact time, and temperature, and used to develop a simple and rapid urinary preconcentration method. The newly developed method was validated with spiked recovery tests that demonstrated the modified rice hull possessed excellent adsorption/desorption characteristics in water matrices. We anticipate that the new modified rice hull adsorbents may be used to support the application of metallomics research in human health and disease as well as preconcentration of trace metals in environmental applications. The detailed methods and findings of this study will be presented at the conference. This study is supported by Missouri S&T Minor Tank Innovation Fund.

Keywords: ICP-MS, Metals, Solid Phase Extraction
Application Code: Clinical/Toxicology
Methodology Code: Separation Sciences
Development of a Novel Bipolar Electrochemically Generated Fluorescence Based Detection Method for Microchip Electrophoresis

Separation and detection of biomolecules, such as reactive nitrogen species and antioxidants, using microchip electrophoresis coupled with amperometric detection (ME-EC) is becoming increasingly popular. ME-EC is compatible with small sample volumes, offers fast analysis times, generates high separation efficiencies, and can easily be miniaturized. However, the limits of detection (LODs) for ME-EC are generally limited to the low micromolar range and are, therefore, not sufficient to quantify analytes that are present at submicromolar concentrations.

Fluorescence is an alternative detection approach that offers picomolar LODs due to low background. However, this approach normally requires the derivatization of the compound prior to analysis, which can diminish selectivity and sensitivity. An alternative is to use a bipolar electrode, where two redox reactions coupled at two opposite poles can be used to convert amperometric current to an optical measurement, such as fluorescence. In this work a novel bipolar electrochemistry/fluorescence based detection method for ME is described to improve the LODs for detect electrochemically active analytes.

In these studies, a 5 cm, 40 \([\text{micro}]\)m x 15 \([\text{micro}]\)m PDMS channel was used for the separation and detection of analytes at the sensor end (ME-EC), while a second flow channel was used for fluorescence reporting. Dichlorodihydro-fluorescein was used as the fluorescence reporter with a laser source at 488 nm. The system was successfully demonstrated with benzoquinone and resazurin as model analytes in the reductive mode.

**Keywords:** Biosensors, Electrochemistry, Electrophoresis, Fluorescence

**Application Code:** Bioanalytical

**Methodology Code:** Electrochemistry
Striving to ultra-high resolution in liquid chromatography (LC), is essential for analysis of the complex samples encountered in today’s research. Since the beginning of the 21st century, tremendous improvements have been made in LC column technology (sub-2 µm porous particles, core-shell particles) and in instrumentation (pressures up to 1500 bar) opening new possibilities in terms of speed and resolution to LC practitioners for tackling complex samples.

A now well-accepted metric of separation power in gradient elution, which is mandatory for samples of high complexity, is peak capacity (nc). With state-of-the-art one-dimensional LC, peak capacities of ca. 600 (in ca. 1 h) can routinely be obtained. It is, however, wishful thinking that such peak capacities are sufficient to separate very complex mixtures. Giddings estimated that an nc of 10.000 is needed to “chromatographically” resolve a sample containing 100 components!

A straightforward approach to increasing nc is bi-dimensional LC or 2D-LC. On-line 2D-LC is divided in heart-cutting LC (LC-LC) and comprehensive LC (LC×LC). LC-LC is applied to better resolve components in selected retention time windows, while in LC×LC, the entire sample is subjected to two separation mechanisms.

The recent developments in 2D-LC will be illustrated with analysis of several complex matrices (omics, (bio)pharmaceuticals, natural products, etc.). Figures of merit will be presented and the ruggedness of 2D-LC using commercial instrumentation will be discussed in relation to its routine application in GMP and QA/QC.

Keywords: Biopharmaceutical, Liquid Chromatography, Liquid Chromatography/Mass Spectroscopy, Natural Products
Application Code: General Interest
Methodology Code: Liquid Chromatography
Capillary LC with Sub-2 Micron Particles: Effects of Column Packing Conditions on Column Morphology and Efficiency

Ultra High Pressure Liquid Chromatography (UHPLC), with operating pressures in the range of 1,000 to 3,000 bars, permits operation of long capillary columns packed with particles between 1 and 2 micron diameter. In a collaborative research project between the Jorgenson group and the Tallarek group, we are determining some of the underlying structural causes of poor column efficiency. We are using this knowledge to guide development of methods to pack increasingly efficient columns. This effort has recently resulted in production of capillary columns 100 cm long packed with 2 micron particles which produce 500,000 theoretical plates at the optimum velocity. This corresponds to a minimum plate height of 2 microns, or one particle diameter. The correlation of several characteristics of the packed bed structure with column performance will be discussed. Based upon this structural information, methods for the slurry packing of micron-sized particles to produce columns of exceptional separation power will be described.

References:


Keywords: Capillary LC, Chromatography, Liquid Chromatography
Application Code: Bioanalytical
Methodology Code: Liquid Chromatography
Abstract Text

Extreme complexity of the glycan mixtures derived from glycoproteins that are extracted from biological fluids and tissues demands utmost from the available analytical tools. The structural and functional diversity of glycoconjugates includes their well-known propensity to form numerous isomers. Since mass-spectrometric (MS) methodologies alone find it difficult to address isomerism, high-efficiency (capillary LC) separations can be beneficial prior to positive identification through tandem MS. Using small-bore capillaries, packed with very small particles, provides the separation media with high numbers of theoretical plates combined with adequate sample capacity to ensure reliable identification through tandem MS. The separation of isomeric glycans can often be accomplished through the use of small HILIC-type or graphitized carbon black spherical particles. Highly efficient capillary LC systems are under development for ultrahigh-pressure operation featuring optimum, “MS-friendly” mobile phase gradients. The analytical merits of these “hyphenated” systems will be demonstrated while featuring N- and O-glycan separations and measurements with the biomedically relevant samples such as cancer serum biomarkers and urinary exosomes. Whereas identification of smaller oligosaccharides has been previously addressed, our more recent investigations concern biological significance of larger tri- and tetra-antennary N-glycans with different fucosyl substitution and sialyl linkages. For a comprehensive coverage of different glycans, it is often advantageous to employ sample enrichment and different derivatization methods.

Keywords: Bioanalytical, Biomedical, Carbohydrates, Chromatography
Application Code: Biomedical
Methodology Code: Liquid Chromatography/Mass Spectrometry
From our early work on the kinetic plot method, we gained insight into how column lengths can be adapted to the requirements of a separation to ensure the best and fastest solution is obtained. This led to the observation that short columns not always result in the fastest separation. Column lengths should rather be chosen such that the required efficiency for a specific separation problem is attained when operating this column length at the maximum available pressure.

Aiming for gains in efficiency or speed of analysis by tailoring the column length to the separation under consideration, we subsequently developed a column coupling solution that allowed changing the length of a column in a fully automated way. This set-up was based on the serial coupling of chromatographic columns using two rotor stator valves with a dedicated groove pattern. We demonstrated that this device could also be used to speed up method development, by using a separation on a short column first to rapidly gain insight into the composition of the sample, and then switching to a longer column to fine-tune the separation. This “variable-length method development strategy” has been applied to pharmaceutical, bio-analytical and environmental samples with varying degrees of complexity.

To also alter the selectivity of a separation method in a flexible way, we have recently modified the automated column coupler to allow the serial coupling of columns with strongly differing stationary phase properties. As a proof-of-principle, we have demonstrated that HILIC and RPLC columns can be coupled in series, and in this way considerably increase the selectivity of a separation, provided adequate means are in place to change the solvent composition in between separations. In the past year, we have been working on solutions that allow this solvent exchange in a fast, flexible and cheap way. With these new developments, we aim to provide solutions for complex samples that can be used in routine applications.

Keywords: Automation, HPLC Columns, Liquid Chromatography/Mass Spectroscopy, Method Development
Application Code: Other
Methodology Code: Liquid Chromatography
In this talk, it will be attempted to make a look into the far future of HPLC, by describing how the ideal liquid chromatography system should look like in terms of packing structure, stationary phase properties and extra-column design. For each item, a theoretical motivation will be given as well as a possible practical approaches to actually fabricate the pursued structure or device. In addition, the expected performance will be related to the current performances, to assess how close to optimality our current systems are.

Results will be corroborated using computational fluid dynamics simulations of the band broadening and the pressure drop in 2-D and 3-D cylinder and sphere packings, as well as experimental results obtained on micro-machined chromatography columns produced using state-of-the-art photolithographic etching.
Polycrystalline electrocatalytic materials, such as platinum, show complex responses when structure-sensitive reactions are addressed. To understand the origin of the different contributions and design more effective electrocatalytic materials, ideal models are required, both from the theoretical and experimental viewpoint. The best experimental model to analyze the surface of polycrystalline platinum, including its nanoparticles, is single crystal reactivity. In this latter case, the surfaces are uniform and a limited, well-known number of sites are involved. For in-situ characterization purposes, the convenient probe reactions are surface confined processes such as those taking place in underpotential deposition or irreversible adsorption at electrolytes of well defined composition. In this way, quantitative analysis of site contribution to reactivity is easily achieved, particularly when series of stepped surfaces are compared. In this framework, examples on the use of single crystals to characterize Pt nanoparticles and its reactivity will be discussed.

Keywords: Adsorption, Electrochemistry, Electrode Surfaces, Voltammetry
Application Code: General Interest
Methodology Code: Electrochemistry
Platinum is still the most important catalyst and electrode material in many applications of electrocatalysis. In this seminar I will discuss our understanding of the surface electrochemistry of platinum in contact with aqueous electrolyte solutions of different pH. First, I will attempt to identify the different surface phases that form on platinum (single-crystal) surfaces as a function of electrode potential. Some first applications of Shell-Isolated Nanoparticle-Enhanced Raman Spectroscopy to the characterization of single-crystal platinum electrodes will be presented. Next, I will discuss the differences between the hydrogen evolution reaction (HER) kinetics on Pt(111) electrodes in acidic and alkaline solution, and present a new explanation for the slow kinetics of the HER in alkaline solution. Finally, I will illustrate how applying negative potentials between -2 and -10 V to platinum leads to new platinum surface chemistry called cathodic corrosion, involving preferential etching and the formation of nanoparticles.

Keywords: Electrochemistry, Electrode Surfaces, Raman Spectroscopy

Application Code: General Interest

Methodology Code: Electrochemistry
The detailed knowledge of the interfacial region at the surface of an electrode is of paramount importance to understand its reactivity. Among the different parameters that characterize the electrified interphase, the potential of zero charge is of great significance. It provides the relation between two essential variables: the potential and the charge. Interfacial studies in electrochemical environment can be done on the basis of the electrocapillary equation. For electroactive materials, such as platinum, the existence of adsorption processes complicates such studies, which should take into account the charge contribution from these processes [1]. This leads to the distinction between total (including adsorption processes) and free charge (the true electronic charge responsible for the electric field at the interphase) [2]. Total charges for platinum electrodes can be obtained using the CO displacement method [3]. Access to the free charge is more elusive and requires extra assumptions. Moreover, study of the interphase as a function of the temperature allows determination of additional thermodynamic properties, such as the entropy of formation of the interphase and the adsorption entropies. Among the different methods to increase the temperature of the interphase, the use of high power laser irradiation allows changing the temperature in a very short time scale. In this case, separation of adsorption processes from pure double layer phenomena can be achieved based on their different kinetic response [4].

Figure 1: cyclic voltammograms and total and free charge curves for a Pt(111) electrode in solutions of different pH. Scan rate: 50 mV/s

References

Keywords: Adsorption, Electrochemistry, Electrode Surfaces, Voltammetry
Application Code: General Interest
Methodology Code: Electrochemistry
Our motivation is to elucidate the impact of structural and reactive heterogeneity at the nanoscale on the macroscale performance of materials for energy conversion and storage. New reactive strategies, materials, and an expanded electroanalytical toolbox are allowing us to discover synergies that enable new concepts for flow batteries, ultra-thin battery interfaces, and electrocatalysis. For example, highly soluble redox active polymers (RAPs) studied in our group have been used for a new type of size-exclusion approach for flow batteries. [1] In these systems, three-dimensional charge transport plays a key role in their performance, but it also imposes limitations to their reactivity that we have tackled through molecular design and through an inspection of their interactions with the different electrolytes.

These studies highlight the importance of elucidating ionic reactivity, thus our group has developed in parallel new tools for following ion transport in real time. In particular, Hg probes for scanning electrochemical microscopy (SECM) that are uniquely sensitive to redox reactivity and ionic fluxes are opening new opportunities in the analysis of battery interfaces, [2] including RAPs and their films. Using these and other tools, we are starting to understand fundamental balances between electronic and ionic reactivity that we hope will have an impact on a various other applications for energy conversion and storage.

References:

Keywords: Electrode Surfaces, Energy, Material Science, Nanotechnology
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Electrochemistry
We describe fundamental studies of single bubbles of H₂, N₂, and O₂ at the surface of Pt nanoelectrodes that are created by electrogeneration of large gas supersaturations at the electrode/electrolyte interface. We present results from "standard pressure-additions" electrochemistry that allows for the direct determination of the Laplace pressure and surface tension of individual nanobubbles as small as 7 nm radius. Our results suggest that the Young-Laplace equation provides a remarkably accurate description of the thermodynamics of high internal pressure bubbles. We also demonstrate that electrochemical measurements of the dissolved gas concentration, at the instant prior to nucleation of an individual nanobubble of H₂, N₂, or O₂ at a Pt nanodisk electrode, can be analyzed using classical thermodynamic relationships (Henry’s Law and the Young-Laplace equation – including non-ideal corrections) to provide an estimate of the size of the gas bubble nucleus that grows into a stable bubble. For example, the measured critical surface concentration of H₂ at the instant of bubble formation corresponds to a critical H₂ nucleus that has a radius of ~3.6 nm, an internal pressure of ~350 atm, and contains ~1,700 H₂ molecules.

Keywords: Electrochemistry
Application Code: General Interest
Methodology Code: Electrochemistry
Targeted cancer therapies are the current focus of cancer drug development. Additionally, expression levels for multiple biomarkers have been shown to correlate with efficacy of chemotherapy, indicating that measuring tumor expression of these proteins could allow chemotherapy to be treated as targeted-therapy. One of the challenges in developing targeted therapies is the identification of patients likely to have clinical benefit. Therefore, it is essential to develop a biomarker assay with good specificity, sensitivity, and predictive value for clinical response.

Using trypsin digestion mapping of recombinant proteins, we identified unique peptides and built quantitative MS-based assays for receptor tyrosine kinase (e.g. EGFR, HER2, MET), proteins involved in immune checkpoints (PD-L1), clinically-actionable gene rearrangements (ALK, ROS1) and sensitive/resistant biomarkers for chemotherapy (e.g. TOPO1, TOPO2A, FRalpha, ERCC1). These assays were multiplexed into a single SRM analysis of 1ug of tumor protein. We quantified these biomarkers in over 400 clinical biopsies in our laboratory and demonstrated reproducibility of the assay over years.

TOPO2A, a biomarker for anthracycline-based therapies, was detected in 74% breast cancer tissues (51/69) ranging from 266-1975amol/ug and 75.8% primary GEC biopsies (47/62) ranging from 318-22550amol/ug. We detected HER2 in 69.6% (48/69) breast cancer and 67.7% (42/62) GEC cancer cases by SRM, ranging from 212-9400amol/ug and 185-9600amol/ug, respectively. 4.4% (3/69) of breast cancer and 12.9% (8/62) of GEC showed HER2-SRM >750amol/ug, a cutoff consistent with gene amplification suggesting that these patients would be candidates for HER2-targeted therapies. ALK and ROS1 proteins, targets of crizotinib/ceritinib, were seen in 1% (1/95) and 2.1% (2/95) of NSCLC tested, respectively.

We quantify the expression of clinically-relevant biomarkers directly in patient tumor tissue, providing information for clinical decision.
[b]Background[/b]
Vitamin D insufficiency has been linked to a wide range of diseases. Certain vitamin D metabolites may have a stronger association with poor clinical outcomes than others. However, for many years, the reproducibility, sensitivity and/or specificity of assays used to quantify those metabolites have been suboptimal. Although significant insights into vitamin D biology have been gleaned from in vitro experiments, the results do not universally translate to patients. To learn about human vitamin D biology from epidemiological studies (including cohort studies and clinical trials), there is an explicit need for well-validated assays that can be translated directly clinical care. Liquid chromatography-tandem mass spectrometric assays relevant to vitamin D metabolism have improved our ability to evaluate human biology on the epidemiological scale.

[b]Methods[/b]
We have developed a multiplexed assay for the quantification of vitamin D metabolites in serum/plasma that uses immunoaffinity purification to enrich for specific molecules of interest. Separately, we have developed a targeted proteomics assay that quantifies serum/plasma concentrations of vitamin D binding globulin and identifies an individual’s genotype. For both, we have improved the throughput of analyses with an off-the-shelf column switching/column regeneration solution from the Waters Acquity family of chromatography systems.

[b]Results and Discussion[/b]
The assays have been used to demonstrate the role of kidney disease in modifying serum concentrations of vitamin D catabolite, to identify patients with CYP24A1 deficiency, and to evaluate intra-individual variability of serum based markers of vitamin D metabolism in vivo. The precision of the assays, even with column switching/column regeneration, is sufficient for clinical research and patient care.

**Keywords:** Clinical Chemistry, Liquid Chromatography/Mass Spectroscopy, Metabolomics, Metabonomics

**Application Code:** Clinical/Toxicology

**Methodology Code:** Liquid Chromatography/Mass Spectrometry
Mass spectrometry can provide precise quantitation of specific target proteins using tryptic signature peptides as surrogate analytes, thereby providing absolute specificity and multiplexability absent in typical immunoassays. The limited sensitivity and throughput of such directed MS assays can be radically improved by affinity enrichment of the signature peptides using anti-peptide antibodies prior to MS measurement (SISCAPA technology). The resulting hybrid immuno-MS approach combines the advantages of conventional immunoassays and MS technology, while eliminating the disadvantages of both. Capture at the peptide level after sample digestion, rather than the whole protein level, eliminates interferences caused by autoantibodies or other protein:protein interactions, and facilitates multiplexing without cross-talk between assays.

Hundreds of polyclonals and ~100 monoclonal anti-peptide antibodies have been developed for SISCAPA use in both research and clinical applications. One such assay, for thyroglobulin at pM levels in human plasma, is now in routine use by multiple large clinical reference laboratories in the US, and a suite of 22 assays for known clinical biomarkers has been successfully applied to longitudinal studies in dried blood spot (DBS) samples.

Longitudinal DBS samples from many individuals demonstrate steady, but unique to each individual, baseline levels of a range of protein biomarkers and profound fluctuations in levels of the acute phase proteins (e.g. CRP, LPS binding protein, mannose binding lectin) in response to such physiological conditions as a common cold, infection or even during different phases of exertive activity. It is anticipated that chronic health conditions that cause more subtle changes in the proteome can be identified and monitored via use of longitudinal DBS samples.

Keywords: Clinical Chemistry, Liquid Chromatography/Mass Spectroscopy, Peptides, Proteomics
Application Code: Bioanalytical
Methodology Code: Mass Spectrometry
Given that enzymes are the effectors of biological action, forward progress in treatment and diagnosis of disease requires the ability to accurately quantify enzyme activity against a backdrop of immense patient variability. While the process to biochemically characterize enzymes is established, much less is known about how to quantify enzyme activity using LC-MS/MS in a high-throughput clinical diagnostics setting. Herein, we report two case studies using LC-MS/MS that illustrate 1) how to establish analyte recovery through a multi-enzyme reaction in a specimen-dependent manner and 2) how to calibrate an enzymatic reaction using a synthetic substrate and relative activity. These studies set the foundation for how to quantify enzyme activity in a clinical setting and thus impact patient health by understanding the biological agents of action.

Keywords: Automation, Bioanalytical, Biological Samples, Mass Spectrometry
Application Code: Clinical/Toxicology
Methodology Code: Mass Spectrometry
Mass spectrometry is increasingly used in clinical and research settings for the measurement of protein concentrations. In most cases, peptides derived from enzymatic digestion of the protein(s) of interest are quantified and used to infer the protein concentration. Although this process can be relatively straightforward, problems can arise if certain factors are not considered in method development. This presentation will describe methods that have been used at the National Institute of Standards and Technology (NIST) for quantification of protein biomarkers such as C-reactive protein and cardiac troponin I and will outline reasons that a one size fits all strategy is very difficult to achieve when dealing with such complex analytes.
Volatile organic compounds (VOCs) found in the human exposome come from external environmental sources and from internal cellular metabolism. Although primarily found in breath, VOCs are also measurable in all bodily fluids including blood, urine, and saliva, as well as from dermal emissions. Furthermore, not all cells contributing to the exposome are human; it is generally accepted that the human gut and the pulmonary microbiome comprise more than 10-times as many cells as those from the human body. As such, if perturbations or patterns of the exposome are to be used as indicators of health state, it is important that their sources are properly attributed. This can have different forms; if a disease such as cancer is affecting the human metabolism, then unrelated changes in the microbiome could confound diagnostic results. Conversely, if the diagnostic test is geared towards fingerprinting a VOCs pattern specific to a particular pulmonary infection, then internal and external influences from human cells, environment, or food could obscure that identification. One approach to reducing such errors is to carefully assess suites of emitted compounds from in vitro cellular systems and from in vivo sampling to get an idea as to “what is unremarkable”. New technologies are now being applied to identify and interpret biological pathways at the cellular level, particularly using bioreactors, microfluidics, and various static-well systems coupled with liquid and gas-phase analytics. This enables the advantages of high-throughput, simplicity, reduction in cost, and avoidance of the ethical concerns inherent to animal model or human research. Challenges remain in data interpretation, and in confidence that in vitro results can be linked back to in vivo outcomes.
The shelf-life of packaged meat can be extended by adopting diverse packaging and storage regimes, including vacuum packaging, modified atmosphere packaging (MAP), chilled or frozen storage. Traditional procedures to determine meat spoilage are microbial analyses in combination with sensory assessments, but these are time-consuming and costly methods, with results typically only available after several days. It has been postulated that volatile organic compounds (VOCs) arising during degradation processes in packaged meat that are subsequently released into the gas-phase of the packaging might be suitable indicators for spoilage. The discovery of specific VOCs that reflect negative quality changes might then be usable in dynamic food freshness indicators (FFIs) of packaged meat to provide producers, retailers and consumers with an indication of whether a product is still palatable. This study presents a novel method to monitor volatile spoilage markers in the headspace of meat in real-time using proton-transfer-reaction mass spectrometry (PTR-MS). The system allows for highly time-resolved release curves, which enable the release kinetics of spoilage markers to be examined in detail in order to gain a better understanding of the temporal processes involved in the spoilage. Furthermore, the storage conditions can be varied to enable a systematic investigation of the effects of different parameters, e.g., modified packaging atmosphere composition, storage temperature, cut of meat, etc. The analytical set-up was tested on different meats (chicken, beef, pork) under diverse storage conditions. Several VOCs were observed to increase during storage, including various groups of compounds. Most VOCs increased exponentially in a similar manner to the typical growth curves of microorganisms (lag phase, exponential phase). The system has potential for extending our knowledge on spoilage processes to address food safety and reduce waste.
Breath-based metabolomics, the analysis of volatile metabolites in breath, promises a non-invasive window into the body to detect metabolic disorders, diseases etc. However, our own metabolism is not the only source of exhaled volatile organic compounds (VOCs). While the impact of exogenous compounds, inhaled or ingested, is actively researched, there is less known on the contribution of our complex bacterial ecosystem, which could even be the stronger source. We demonstrate the complexity of bacterial emissions, by observing a biologically simple system.

In a biopharmaceutical fermenter genetically modified bacteria produce modern drugs. With one genetically defined strain, under precisely controlled environmental conditions, these systems are biologically as simplified as possible. The aeration flow providing oxygen, takes up volatile metabolic byproducts. This allows monitoring volatile emissions by analyzing the offgas using a Proton-Transfer-Reaction Mass-Spectrometer (PTR-MS) – a highly sensitive, online monitor for VOCs.

In this simple system we find more than 70 different VOCs with distinct variation over the course of a fermentation, and their interpretation is complex. A direct correlation to specific metabolic activity is only possible for a few VOCs. We show that such direct correlations are only possible for metabolites with low solubility. As a solution, we introduce a mathematical model to calculate the relevant metabolic production rate, independent of the solubility, from the dynamically measured offgas concentration. From these findings we can draw important conclusions for both, monitoring and control of pharmaceutical fermentations, as well as the interpretation of bacterial contributions to human breath.

Keywords: Biotechnology, Chemical Ionization MS, Process Monitoring, Volatile Organic Compounds
Application Code: Biomedical
Methodology Code: Process Analytical Techniques
The U.S. Environmental Protection Agency is tasked with evaluating the human health, environmental, and wildlife effects of over 80,000 chemicals registered for use in the environment and commerce. The challenge is that sparse chemical data exists; traditional toxicity testing methods are slow, costly, involve animal studies, and cannot keep up with a chemical registry that typically grows by at least 1000 chemicals every year. In recent years, High Throughput Screening (HTS) has been used in order to prioritize chemicals for traditional toxicity screening or to complement traditional toxicity studies. HTS is an in vitro approach of rapidly assaying a large number of chemicals for biochemical activity using robotics and automation. However, no method currently exists for screening volatile chemicals such as air pollutants in a HTS fashion. Additionally, significant uncertainty regarding in vitro to in vivo extrapolation (IVIVE) remains. An approach to bridge the IVIVE gap and the current lack of ability to screen volatile chemicals in a HTS fashion is by using a probe molecule (PrM) technique. The proposed technique uses chemicals with empirical human pharmacokinetic data as PrMs to study toxicity of molecules with no known data for gas-phase analysis. We are currently studying the xenobiotic-metabolizing enzyme CYP2A6 using transfected BEAS-2B bronchial epithelial cell line. The CYP2A6 pathway activity is studied by the formation of cotinine from nicotine and tert-butyl alcohol (TBA) from methyl tert-butyl ether (MTBE). Modulation of the formation of metabolites due to the introduction of a toxicant indicates an alteration of cellular homeostasis. To measure perturbations in metabolite formation, cotinine is analyzed by standard liquid phase immunoassay (ELISA), while TBA is measured using gas-chromatography-mass spectrometry (GC-MS).

This is an abstract of a proposed presentation and may not reflect official US EPA policy.

**Keywords:** Clinical/Toxicology, GC-MS, Headspace, High Throughput Chemical Analysis

**Application Code:** Clinical/Toxicology

**Methodology Code:** Gas Chromatography/Mass Spectrometry
The analysis of panels of molecular biomarkers and cellular phenotypes offers valuable diagnostic and prognostic information for clinical decision making. Robust, practical platforms that detect low levels of biomolecules and rare cells are urgently needed to advance medical care by diagnosing and predicting the progression of cancer and other disease states. 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 (1). In addition, we have worked towards integrating nanomaterials-based electrodes into a chip-based platform to facilitate multiplexed analysis in a robust, practical format. Recently, we have developed assays that are able to detect nucleic acids, proteins and small molecules, with universally high sensitivity levels (2). Our efforts to use these components to detect markers in clinical samples to develop tests for infectious disease diagnosis, oncological management and transplant medicine will be featured in this lecture (3-6).

What can we achieve when we start to dramatically reduce the size scale of polymeric ion sensors? This talk will show how nanometer sized films and spheres can give rise to new functionalities that are not possible with macroscale sensing films.

One direction focuses on the development of optical ion sensors based on bulk extraction phenomena, but that start to approach single ion response characteristics as their size gets dramatically reduced. In one such direction, the fluorescent transducing molecule is tethered to the surface of the sensor phase, resulting in a localized partitioning of the solvatochromic dye. Fundamental limitations as well as exciting new application possibilities will be discussed.

With electrochemically addressable sensing films of ultra-thin dimensions, one may achieve a localized equilibration at every applied potential during a linear sweep. This makes it possible for the first time to measure the activity of multiple ionic analytes at the very same location. Experiments and simulations will be presented, along with the discussion of potential new applications using this methodology.

Keywords: Bioanalytical, Fluorescence, Sensors
Application Code: Bioanalytical
Methodology Code: Sensors
Nanopore techniques have proven to be useful tools for single-molecule analysis. Protein nanopores offer an inexpensive, label-free method for analysing single oligonucleotides. The sensitivity of the approach is largely determined by the characteristics of the pore-forming protein employed and typically relies on nanopores that have been chemically modified or incorporate molecular motors. Here, we show that a wild-type (WT) aerolysin nanopore can resolve individual short oligonucleotides that are 2 to 10 bases long. The sensing capabilities are attributed to the geometry of the aerolysin and the electrostatic interactions between the nanopore and the oligonucleotides. We also show that the WT aerolysin nanopores can distinguish individual oligonucleotides from mixtures and can monitor the stepwise cleavage of oligonucleotides by exonuclease I. More important, WT aerolysin shows an unexpected capability to quantify the subpicomolar level of DNA oligomer and distinguish single-nucleotide variations from the mixtures. Therefore, aerolysin will play a significant role in the future nanopore-based single-molecule analysis and promote the conversion of nanopore techniques to practical applications.

References

Keywords: Bioanalytical, Biosensors, Electrochemistry, Nanotechnology
Application Code: Bioanalytical
Methodology Code: Electrochemistry
Measuring molecular interactions with membrane proteins and quantifying the interaction kinetics are critical for understanding many cellular processes, for validating biomarkers, and for screening drugs. This is because membrane proteins are responsible for many important functions of cells, including communication with other cells, sensing the surrounding environment, and transporting molecules and ions in and out of cells. They also comprise nearly 60% of current drug targets. However, developing such a capability has been a difficult challenge, especially for small molecules, because most traditional binding kinetics measurement technologies are based on the detection of molecular mass, which diminishes with the size of the molecule. Small molecules are the most important forms of drugs, accounting for over 70% of all the drugs developed to date. We will discuss several new label free optical technologies (plasmonics and digital imaging techniques) that can detect and quantify the interactions of both larger and small molecules with membrane proteins on intact cells.
Advances in measurement science have seen a progressive reduction in sample size to the point that single-molecule measurements are today commonplace. A new generation of sensors is expected that perform quantitative analysis by measuring many single-molecule events. This talk discusses the challenges and opportunities presented by quantitative single-molecule sensors. It will then cover three technologies developed in our laboratory that allow the detection and quantification of single molecules. The first is a smart plasmonic sensor that can reversibly trap a single molecule at hot spots for rapid single-molecule detection using surface enhanced Raman spectroscopy. The second looks at the application of solid state nanopores and magnetic nanoparticles for the quantitative analysis of rare protein species to give devices with ultralow sensitivity but rapid response time. The final technology that is described is using single molecule localisation microscopy to monitor bioaffinity reactions between surface bound proteins and antibodies.
Top-down proteomics provides important information regarding multiple post-translation modifications on the same protein, but its utility has been hampered by low chromatographic resolution. Submicrometer particles, which give improved efficiency, are promising for top-down proteomics. Current RPLC bonded phases were designed to work with TFA, which strongly suppresses ionization when MS is used for detection. Innovations in the bonded phase are also explored.

Keywords: Chromatography
Application Code: Bioanalytical
Methodology Code: Liquid Chromatography/Mass Spectrometry
A new series of materials for HIC (hydrophobic interaction chromatography) retains proteins adequately in concentrations of ammonium acetate low enough to be compatible with direct mass spectrometry (MS). Proteins elute with native structure intact, simplifying the mass spectra. Various monoclonal antibodies (mAbs) and antibody-drug conjugates (ADCs) were analyzed via this method. The behavior of mAbs was affected by the pH. The initial salt concentration had less effect, a distinctive feature of the new HIC materials. This method should facilitate the analysis of protein variants by online MS without the need for fraction collection and desalting.
Integration of Liquid Chromatography and Mass Spectrometry in Proteomics

A Promising Alternative to SWATH: Ionstar for In-Depth, Large-Scale and Reproducible Quantification with High Accuracy/Precision and <1% Missing Data

In-depth and reproducible protein measurement, is essential for pharmaceutical/clinical proteomics, but challenging owing to suboptimal sensitivity, accuracy, reproducibility and false-positive-biomarker-discovery. Moreover, severe missing values often precludes reliable analysis of large cohorts. MS2-DIA approaches were developed to lower missing values but often with limited depth. Here we describe an Ionstar strategy that achieves in-depth and reproducible quantification in large cohorts with high accuracy, precision and extremely-low missing data. Highly reproducible and efficient sample preparation and chromatographic strategies were employed to enable robust analysis of many samples; a selective trapping with 100-cm column was used for separation and quantitative MS1 signal was acquired@120k resolution by an Orbitrap Lumos for high sensitivity and selectivity. Quantifiable proteins were filtered under stringent criteria and then processed with IonStar, including chromatogram alignment, feature generation and IC extraction, data filtering, merging, normalization and aggregation. The data pipeline was thoroughly compared to popular methods including spectral counting, Proteome-Discoverer, OpenMS and Maxquant, using a 5-group, 20-replicates benchmark sampleset. IonStar showed by far the most in-depth analysis, best accuracy, intra-group-variation (~5%CV vs.11-18% by others) and lowest missing data (<0.2% vs.18-44%) and false-positive-biomarker-discovery-rate(<4% vs.7-31%), with quantified protein abundances spanning ~5.8 orders of magnitudes (vs.3.2-4.4). As a proof of concept, this strategy was applied in the analysis of a large biological cohort: investigation of the temporal effects of three chemotherapy regimens on pancreatic cancer(N=48), where 5049 unique proteins were quantified in all 48 samples without any missing data. The Ionstar is a promising alternative to SWATH, with much higher proteomic coverage and comparable or lower missing data levels.

Keywords: Liquid Chromatography, Mass Spectrometry, Proteomics, Quantitative
Application Code: Genomics, Proteomics and Other ’Omics
Methodology Code: Liquid Chromatography/Mass Spectrometry
Nutrient restriction (NR), or caloric restriction, has many documented health benefits, including extended healthy life span and reduced disease risk. NR is also reported to reduce cancer initiation and progression and render cancer cells more sensitive to chemotherapy. While there is tantalizing preliminary evidence that combining NR with chemotherapy treatment can selectively destroy cancer cells, the molecular pathways that underwrite the process are poorly understood. To help design intelligent molecular therapies for colorectal cancer involving NR, we are investigating the proteomic changes that result from NR. To mimic human tumors, we are using three dimensional cell culture models, also known as spheroids, which mimic primary tumors.

We subjected colon cancer spheroids to NR, depriving them of serum or glucose, and then examined the global quantitative proteomic changes by liquid chromatography high resolution tandem mass spectrometry. To investigate the phenotypic changes in the cancer cells, we performed flow cytometry analysis to determine the numbers of cells undergoing apoptosis and autophagy with each form of NR.

We identified numerous proteins that were differentially regulated by restriction of either serum or glucose. All proteins that were up regulated by one form of NR were also up regulated with the alternative form of NR. The identical pattern was observed for all of the down regulated proteins. Some of the proteins that were up regulated by both glucose and serum restriction include sirtuin 1 (SIRT1) and protein inhibitor of activated STAT1 (PIAS1). Conversely, both glucose and serum restriction caused down regulation of multi-drug resistance protein (MRP1) and Zinc finger and BTB domain-containing protein 7A (ZBTB7). We also determined that NR causes lower apoptosis rates and higher autophagy rates. These studies provide an initial framework to help establish rational molecular therapies for colorectal cancer involving NR.
A deep proteome profiling including the identification, characterization, and quantification of "proteoforms" arising from genetic variations, alternative RNA splicing, and post-translational modifications is essential to understand disease mechanisms and discover therapeutic targets. Top-down mass spectrometry (MS)-based proteomics analyzes intact proteins and has unique advantages for the comprehensive analysis of proteoforms. However, the extreme complexity of the proteome, which is comprised of thousands of proteins corresponding to millions of proteoforms, presents a significant challenge in top-down proteomics. Consequently, multi-dimensional liquid chromatography (MDLC) strategies, which can be coupled to the mass spectrometer and are amenable to automation, are highly desired. However very few MDLC approaches have been developed to separate intact proteins for use in top-down proteomic analyses.

In the past few years, our lab have been developing new materials and novel strategies to separate intact proteins in the MDLC mode. We have identified ammonium tartrate as a new MS-compatible salt for hydrophobic interaction chromatography (HIC). We have developed a novel 3DLC strategy by coupling HIC with ion exchange chromatography (IEC) and reverse phase chromatography (RPC) for intact protein separation for top-down proteomics. Recently a series of more hydrophobic HIC materials that can retain proteins have been introduced using MS-compatible concentrations of ammonium acetate. Our current focus is to develop a new size exclusion chromatography (SEC) strategy and couple it with HIC, IEC and RPC to achieve a deep proteome profiling. We are applying these MDLC strategies to decipher the proteoforms in the human heart proteome.
The State of Colorado, like so many other states, has faced many challenges while navigating the new world that is legalized cannabis. One of the more unique challenges is the regulation of analytical cannabis laboratories. New testing methodologies in an incredibly complex matrix, the lack of suitable proficiency testing and accepted industry standards, and limited scientific evidence demonstrating the toxicity of various contaminants in cannabis have made development of testing regulations a difficult endeavor.
The cannabis industry is federally unregulated and in many cases under-regulated by states in which medicinal and/or adult consumption is sanctioned. State regulatory bodies are increasingly favoring mandatory product testing for such things as constituent potency, solvent residue, pesticide residue, mycotoxin, and microbiologic contaminants. Cultivators and distributors are being pressed to have their products analyzed, yet there are no standard or consensus methods available to the cannabis industry. Analytical technologies used for this industry are no different than those used in other applied areas. The fundamental difference, however, is about technical competence. The cannabis industry continues to witness good analytical methodologies performed by inadequately trained personnel and used for unintended purposes, compromising the integrity of test results. This presentation will provide a brief overview of analytical trends in the cannabis industry, the challenges analysts and regulatory bodies continue to confront, and discuss the basic framework and benefits of moving testing laboratories toward ISO/IEC 17025 accreditation.

Keywords: Biomedical
Application Code: Biomedical
Methodology Code: New Method
It's Legal! Now What? The State of Sample Analysis in the Era of Legal Cannabis

State Regulatory Laboratory Perspective

The Colorado Department of Agriculture Biochemistry Laboratory conducts regulatory testing of recreational and medical marijuana for pesticide residues and industrial hemp for THC content. Regulatory testing is an inherently governmental function and in Colorado the CDA Biochemistry Laboratory is the primary testing laboratory. Commercial testing services for the cannabis industry are available but in a patchwork fashion. Due an executive order issued by the Governor’s Office essentially requiring "zero tolerance" for pesticides in marijuana, and regulatory enforcement of the State's pesticide application law, CDA and the marijuana industry currently have an adversarial posture. Because of past and ongoing litigation with the cannabis industry, it would be highly unlikely that CDA would rely on any test results supplied by commercial laboratories serving this industry. However, such results may serve a purpose in terms of "provisional testing". CDA is currently involved in serving on state-industry work groups to assist in developing testing strategies for monitoring and compliance.

Keywords: Agricultural, Biopharmaceutical, Natural Products, Pesticides
Application Code: Regulatory
Methodology Code: Chemical Methods
There is a general misconception that Cannabis laboratories are wildly profitable and very easy to operate. The realities of operating a modern Cannabis laboratory are challenging as well as complicated. Cannabis laboratories operate within a 4-6% profit margin because of the expensive nature of the laboratory business. Success ultimately depends on a laboratory operator’s creativity and agility as he or she deals with the shifting sands of local and state regulations, increasing taxes, absence of banking support, lack of traditional financing, increased operating costs, and of course, breaking federal law. Professional expertise is required to analyze and evaluate results from both chemistry and microbiological backgrounds. State of the art equipment is utilized and maintained under ISO accreditation standards for redundancy and reliability. The diversity of forms further complicates the industry requiring many separate SOPS to run a full service laboratory. The work of laboratory associations, like the ACCL, have begun to organize and work together for a unified voice regarding selection of quality measures. Opportunities for improvement will be discussed.

Keywords: Biomedical, Drug Discovery
Application Code: Biomedical
Methodology Code: Laboratory Informatics
There is a good deal of uncertainty about how to approach the analysis of cannabis and cannabis related products. The matrices are complex, the target list is changing and the possibility of additional pesticides or adulterants is constant. Adequate monitoring of these samples requires at least some level of screening analysis to look for non-target compounds. In this presentation, the results from the analysis of samples using a QTOF to analyze cannabis samples will be presented. The discussion will include a description of the instrumentation that was used to acquire the data, the data analysis workflow to identify both targeted and non-targeted compounds and the results for a variety of matrices.

Keywords: Drugs, Identification, Liquid Chromatography/Mass Spectroscopy, Pesticides
Application Code: General Interest
Methodology Code: Liquid Chromatography/Mass Spectrometry
Method Development Strategies for Two-Dimensional Liquid Chromatography Separations – Small an

Two Dimensional Liquid Chromatography Applied to the Characterization of Monoclonal Antibodies and Antibody-Drug Conjugates

Monoclonal antibodies (mAbs) have emerged as important therapeutics for the treatment of life-threatening diseases like cancer and autoimmune diseases. The successes of mAbs have triggered the development of various next generation formats. In oncology, antibody-drug conjugates (ADCs) are particularly promising, since they synergistically combine a specific mAb linked to a biologically active cytotoxic drug via a stable linker. The promise of ADCs is that highly toxic drugs can selectively be delivered to tumor cells thereby substantially lowering side effects as typically experienced with classical chemotherapy.

Monoclonal antibodies have a complexity far exceeding that of small molecule drugs, hence, unraveling this complexity represents an analytical challenge. This challenge becomes even bigger in case ADCs are considered since the heterogeneity of the initial antibody is superimposed with the variability associated with the cytotoxic drug conjugation strategy. The current lecture will highlight the power of two dimensional liquid chromatography in hyphenation to high resolution mass spectrometry for the characterization of mAbs and ADCs. Both multiple heart-cutting LC-LC, applied at protein level, and comprehensive LCxLC, applied at peptide level, will be discussed.

Keywords: Biopharmaceutical, Liquid Chromatography/Mass Spectroscopy, Peptides, Protein
Application Code: Pharmaceutical
Methodology Code: Liquid Chromatography/Mass Spectrometry
It is challenging to have one single analytical method to separate all the impurities of a therapeutic peptide due to their complicated and often similar structures. Health authorities often require an orthogonal method to verify peak purity in the primary method as well as to monitor potential degradants which might co-elute with the active pharmaceutical ingredient (API) or other impurities under primary method conditions. Two-dimensional LC (2D-LC) has much higher peak capacity and resolving power than traditional one-dimensional HPLC, making it an ideal tool for peak purity verification. With commercial 2D-LC instruments becoming available recently, more routine application of 2D-LC in industrial labs is feasible. One isocratic method and one gradient method were developed to assess the purity/impurity of a therapeutic peptide. In this presentation, we will discuss the use of commercial 2D-LC to support method development for this peptide. Agilent 1290 Infinity Solution 2D-LC with multiple peak parking capability was used to verify the API peak purity in a stressed sample which was analyzed using a one dimensional (1D) LC method. Using multiple heart-cutting 2D LC mode, impurities co-eluted with the peptide in the 1D separation were successfully separated from in the second dimension. However, the peak shape in the second dimension separation was distorted due to sample matrix effects. A Waters 2D-LC system equipped with online peak trapping and online dilution was also used to successfully resolve co-eluted impurities from API using heart-cutting 2D LC mode. The peak shape of second dimension separation was significantly improved and comparable to the one dimensional gradient separation of this peptide. These findings lead us to believe that dilution of the transferred fraction, so that it contains less organic solvent than the 2D eluent, is essential for successful 2D LC separations of peptides, which agrees with previous findings.

Keywords: HPLC, Pharmaceutical, Separation Sciences
Application Code: Pharmaceutical
Methodology Code: Liquid Chromatography
Method Development Strategies for Two-Dimensional Liquid Chromatography Separations – Small an

effects of method development decisions on the quantitative performance of two-
dimensional liquid chromatography

With the increasing availability of commercial instrumentation for two-dimensional liquid chromatography (2D-LC) there
is high potential for 2D-LC methods to significantly improve upon the capabilities of 1D-LC, and even displace 1D-LC for
routine analyses in some application spaces. However, for this potential to be fully realized, the quantitative performance
of 2D-LC must be as good or better than 1D-LC, as measured by metrics including accuracy, precision, and detection
limits. As we study these issues in more depth we are finding that these performance measures are critically dependent
on method development decisions ranging from stationary phase choices in the two separation dimensions to the extent
of filling of sample loops during the transfer of first dimension effluent to the second dimension column.

In this presentation we will summarize our current understanding of the factors that most strongly affect the quantitative
performance of 2D-LC. Specifically, we will propose method development strategies that can be used to: 1) improve the
precision of peak area measured at the second dimension detector; and 2) improve detection limits at the outlet of the
second dimension column while maintaining the separation of sample components for which the second dimension is
critically important to the overall 2D resolution. Although more work remains to be done, it is our view that the
quantitative performance of 2D-LC is becoming increasingly respectable and should be a viable option even for routine
analyses and in regulated environments.

Keywords: Biological Samples, Food Science, Liquid Chromatography, Pharmaceutical
Application Code: Pharmaceutical
Methodology Code: Liquid Chromatography
Method Development Strategies for Two-Dimensional Liquid Chromatography Separations – Small an

Two Dimensional Liquid Chromatography for mAb’s: Expanding the Analytical Toolkit for Product and Process Characterization

Recent clinical and commercial advancements in monoclonal antibody based immuno-oncology therapeutics such as Keytruda® have initiated expanded investment within the Biopharmaceutical industry. This requires improving analytical tools to support structure function characterization of these novel drugs.

Traditional release and characterizing testing of such proteins requires multiple analytical methods for biochemical, biophysical, and potency testing. More specifically, primary structure characterization typically relies on a number of different HPLC techniques such as protein A (Pro A), ion exchange (IEX), size exclusion (SEC), reverse phase (RP) and hydrophobic interaction (HIC) applied in single dimension separations.

Two-dimensional liquid chromatography (2D-LC) is being considered to develop multi attribute methods (MAM) to reduce the analytical burden. 2D-LC is a powerful tool for analyzing highly complex samples such as clarified media, cell culture and monoclonal antibodies (mAb’s). Automated transfer of first dimension effluents to a second dimension column provides the ability to characterize multiple product critical quality attributes (CQA’s) within a single analytical run. Advances in 2D-LC instrument software for both method control and data analysis have enabled expanded applications of 2D-LC in Bioprocess Development. This work describes the application of 2D-LC to process and product characterization studies of monoclonal antibodies. Additionally, initial efforts to apply 2D-LC as process analytical technology (PAT) will also be discussed.

Keywords: Biotechnology, Chromatography, Liquid Chromatography, Protein

Application Code: Pharmaceutical

Methodology Code: Liquid Chromatography
Two dimensional liquid chromatography (2D-LC) has revolutionized much of the pharmaceutical industry, but despite the advantages of 2D-LC, there is currently little to no work in demonstrating the suitability of these 2D-LC methods for use in a quality control (QC) environment for good manufacturing practice (GMP) tests. This lack of information becomes more critical as the availability of commercial 2D-LC instrumentation has significantly increased, and as more testing facilities begin to acquire these 2D-LC capabilities, it is increasingly important that the transferability of developed 2D-LC methods be assessed in terms of reproducibility and performance across different laboratories worldwide. The work presented here focuses on the evaluation of a heart-cutting 2D-LC method used for the analysis of a pharmaceutical material, where a key, co-eluting impurity is resolved from the main peak and analyzed in the second dimension. In this method, linearity, accuracy, precision, repeatability, and sensitivity are assessed along with inter-day, analyst-to-analyst, and lab-to-lab (instrument-to-instrument) assessments. Additionally, special focus is devoted towards unique 2D-LC critical method attributes and considerations that transcend conventional method validation qualifications. The 2D-LC method attributes are evaluated for their recovery, peak shape, and resolution of the two co-eluting compounds in question on the second dimension. A design-of-experiments (DOE) approach was taken in the collection of the data, and the results were then modeled in order to evaluate method robustness using statistical modeling software. The results of this validation study demonstrate that the 2D-LC method is accurate, sensitive, and robust and is suitable for QC testing with good method transferability across different testing laboratories.

Keywords: HPLC, Pharmaceutical, Separation Sciences, Validation
Application Code: Pharmaceutical
Methodology Code: Liquid Chromatography
Spherical nucleic acids (SNAs) are an emerging class of nanoparticles consisting of a dense shell of oligonucleotides conjugated to a spherical nanoparticle core. The SNA architecture gives rise to unique properties different from linear nucleic acids, including rapid cellular uptake and increased resistance to nuclease degradation. In addition to being potent gene regulation agents, SNAs are becoming promising new components of cancer vaccines. Indeed, SNAs made of immunomodulatory nucleic acid sequences and carrying tumor-specific antigens activate the immune system to selectively kill tumor cells. The oligonucleotides on the surface of the SNAs activate the innate immune system through toll-like receptors (TLRs) in antigen-presenting cells (APCs). These activated APCs then mature and activate effector T-cells to target and kill tumor cells displaying the cancer antigen. Furthermore, novel SNA cancer vaccines have been developed in which a tumor specific antigen is hybridized to the SNA surface or encapsulated within the liposomal core. This leads to increased co-delivery of oligonucleotide and antigen to the same APC resulting in increased T-cell activation, which translates to three times higher specific killing of tumor cells compared to oligonucleotide-antigen mixtures. With mouse lymphoma models, treatment with SNA vaccines result in a decreased tumor volume and increased survival compared with free antigen. Currently, SNA cancer vaccines are being developed against prostate cancer, melanoma, lymphoma and triple negative breast cancer.

Keywords: Biomedical, Biopharmaceutical, Drug Discovery, Nucleic Acids
Application Code: Nanotechnology
Methodology Code: New Method
Abstract Text

Raman spectroscopy is a widely used analytical method capable of providing valuable information about the chemical structure and composition of molecules. Graphitic nanomaterials possess distinctive Raman signatures, which make them ideal for sensitive Raman detection and imaging. Noble metal graphitic nanocapsules have been designed and utilized for different biomedical applications. We fabricated corrosion-resistant, water-soluble, and graphene-protected AgCu nanoparticles (ACG). Such stable ACG have been utilized for cell labelling, rapid Raman imaging and SERS detection. We also developed a graphene-isolated-Au-nanocrystal (GIAN) nanostructures. Multimodal cell imaging and NIR photothermal-enhanced chemotherapy have been realized with such GIAN platform. Moreover, aptamers were functionalized on GIANs via simple, but strong \( \text{C} \text{O} \text{H} \text{C} \text{O} \) interaction to realize targeted imaging. We also explored the cellular uptake mechanism of the GIAN by monitoring the intrinsic Raman and two-photon luminescence signals of GIANs. The mechanism of cellular uptake in these GIAN nanostructures is determined by the presence or absence of aptamer modification. The unique properties of graphitic nanocapsules are not compromised by these nanocapsular structures, which opens new opportunities for specific molecular recognition, gene therapy and nanomaterials fabrications.

Keywords: Bioanalytical, Imaging, Material Science, Surface Enhanced Raman Spectroscopy

Application Code: Bioanalytical

Methodology Code: Vibrational Spectroscopy
In recent years, the use of nanoparticles has advanced from its original utilization to study cells in vitro to application in animal models for cancer diagnostics and therapy. No such advances have been reported concerning the number one killer, heart disease. Here we report on in vivo photon based diagnostics as well as therapy for both diseases. It is well known that the chemistry of the extracellular tumor environment differs from that of healthy tissues in two major respects: hypoxia, i.e. lower oxygen level, and acidosis, i.e. lower pH. Very recently, drastic changes in the concentrations of physiological ions have also been documented. These changes may seriously affect the efficacy of therapy, e.g. radiation therapy, photodynamic therapy, chemotherapy and immunotherapy. It would thus be advantageous for precision medicine to have this information. We have shown how nanoparticle based photoacoustic imaging can, non-invasively, quantify tissue oxygen, pH as well as of physiological ions, in real time and spatially resolved. Notably this novel photon and ultrasound based chemical imaging technique overcomes the light penetration depth problem of the conventional optical imaging modality and is not only non-invasive but also relatively inexpensive. Regarding novel uses of photomedicine, photodynamic therapy (PDT) has been long used clinically for skin cancers. Previous animal model tests have also indicated its potential use for internal organs and even brain cancer. We now show that PDT can also serve to fix arrhythmia, possibly the most critical aspects related to heart disease. In vivo tests on rodent models as well as on large animal models (sheep), have demonstrated that cell selective photoablation has major advantages compared to the traditional modes of arrhythmia ablation treatment. The latter approach is based on the use of myocyte targeted photoactive nanoparticles. Notably, this novel application of PDT to heart disease requires ultra-small nanoparticles (below 10 nm), in contrast to the photodynamic nanoparticles used for the treatment of cancer, which can be of the order of 100 nm.
Cell types, both healthy and diseased, can be classified by the inventories of proteins present and absent on their surfaces. Here, we report two DNA based nanostructures for biological recognition and mimicking. The first one is the design and implementation of programmable DNA-based logic circuits that can assess an inventory for individual cells and respond based on multiple parameters. These programmable nanostructures combine aptamers that detect specific surface proteins with "toehold" DNA catalysis to create a biocircuit that, via a series of AND, OR and NOT logic decisions, creates an output that depends on what proteins are present and absent on that individual cell. The second one is the step-by-step construction of a prototype mimic of the acquired, or adaptive, immune system (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 biological reaction networks and molecular devices. Both systems are made of DNA strands and can be sued for biological recognition and function.

Keywords: Bioanalytical, Biomedical, Biotechnology, Fluorescence
Application Code: Biomedical
Methodology Code: Fluorescence/Luminescence
### Nanomedicine, From Diagnostics to Large Animal Therapy

**Abstract Title**: Nanomedicine for Functional Imaging and Therapy of Brain

**Primary Author**: Paras Prasad  
**Institution**: SUNY at Buffalo

#### Abstract Text

We introduce a single multifunctional core–multiple shells nanoparticle platform serving as an optical probe as well as contrast agents for photoacoustic and magnetic resonance imaging for molecular, structural and functional imaging of brain. It can be equipped with targeting and the ability to cross the blood brain barrier (BBB) in a systemic delivery to reach a specific intended site, producing a 3D region-specific mapping of brain activity, and delivering a genetic material or a drug for treatment of a brain disease or enhancing its cognitive state. The same nanoparticles act as optical nanotransformers to convert externally incident, skull penetrating near IR light noninvasively on site and on demand, into a blue light to be absorbed by channel rhodopsin to effect optogenetic control of neuronal activities in specified sites of the brain, providing an effective intervention/augmentation strategy to enhance the cognitive state for futuristic vision of super human capabilities. We are also pursuing applications to brain Injury and concussions. We also use another formulation, biodegradable theranostic polymeric photonic nanoparticles that cross BBB and efficiently deliver a therapeutic agent to treat central nervous system diseases such as glioblastoma, simultaneously providing optical tracking of drug delivery and release. This combined imaging enabled region specific diagnostics and therapy (hence theranostics) will be a major breakthrough in understanding and treating neurological diseases such as Alzheimer’s disease, Parkinson’s disease, and mental retardation/autism.

**Keywords**: Biomedical, Imaging, Nanotechnology, Optogenetics  
**Application Code**: Biomedical  
**Methodology Code**: Microscopy
Novel Approaches in Optical Biological Imaging and Bioanalytical Analysis

Ln3+ Based Nanoparticles and Near-Infrared (NIR) Quantum Dots for Optical Bioimaging

We have two main research thrusts in the field of colloidal nanomaterials and their applications. The colloidal stability is typically obtained by a “soft” layer of organic molecules. One thrust is based on Ln3+ doped NaLnF4 nanoparticles for optical imaging. To this purpose, we have developed synthesis techniques to make core and core-shell architectures which allows the optical properties to be optimized. Additionally, we do surface modifications by organic chemistry to impart compatibility with biological media, e.g. pH buffers and serum-enhanced growth media, and to impart binding specificity (e.g. through antibodies).

Similarly for the second thrust, we have developed synthesis routes for high quality PbS(e) based quantum dots with emissions in the near-infrared (NIR) for optical bio-imaging. Also here, core-shell architectures have clear advantages in terms of stability, quantum yield of the photoluminescence, etc.

The synthesis and basic characterisation of the nanoparticles and quantum dots will be discussed, with some emphasis on the challenges to prove the actual formation of core-shell structures. The main focus of the talk is on their surface modification and use as optical probes for bioimaging.

Abstract Text

Primary Author
Frank Van Veggel
University of Victoria

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Abstract Title
Ln3+ Based Nanoparticles and Near-Infrared (NIR) Quantum Dots for Optical Bioimaging

Date: Monday, March 06, 2017 - Afternoon
Time: 01:35 PM
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Symposia

Session Title
Novel Approaches in Optical Biological Imaging and Bioanalytical Analysis

Application Code: Nanotechnology
Methodology Code: Biospectroscopy

Keywords: Biospectroscopy, Fluorescence, Luminescence, Material Science
Novel Approaches in Optical Biological Imaging and Bioanalytical Analysis

Near-Infrared Emitting Lanthanine-Containing Metallacrowns as Novel Imaging Agents for Optical Cellular Biological Imaging

A large number of advanced detection techniques and methodology require the unique spectroscopic properties of lanthanide(III) ions. In particular, their ability to generate characteristic sharp emission bands in the near-infrared (NIR) ranges has a growing interest in view of the exponentially increasing number of applications in bioanalysis and optical imaging.[1] Near-Infrared (NIR) optical imaging has a great research and clinical potential to dramatically improve diagnosis in real time imaging experiments[2]. NIR photons that can cross deeply into tissues for non-invasive investigations and allow for improved detection sensitivity due to the absence of native NIR luminescence from tissues and cells. The main requirement to generate lanthanide emission is to sensitize them with an appropriate chromophore. As of today, the main limitation lies in the low number of photons emitted by the current lanthanide complexes and nanomaterials. We have recently demonstrated that Zn16Ln metallacrowns (MC) obtained by the self-assembly of Ln(III), Zn(II) ions and the chromophoric quinaldichydroxamic acid (quinHA) ligand, is an innovative approach allowing the precise localization of lanthanides at a predetermined and shielded position to achieve high quantum yields and long luminescence lifetimes.[3] In addition, such MCs possess very good photostability. Here, we expand this strategy to Zn16Ln MCs assembled from derivatives of pyrazinehydroxamic acid with the goal to improve biocompatibility and further shift excitation wavelength towards the visible/NIR range. Examples of applications of these MCs in optical microscopy experiments in cells will also be presented here.

Acknowledgement: The research leading to these results have received funding from the European Community's Seventh Framework Programme (FP7/2007-2013) under grant agreement n° 611488.

Keywords: Fluorescence, Near Infrared
Application Code: Bioanalytical
Methodology Code: Near Infrared
There is increasing emphasis on biomedical devices that incorporate graceful or catastrophic degradation into their designs. Whereas degradation is usually needed to allow clearance of the foreign object from tissues or from circulation, the degradation of some nanostructures can be harnessed to form new, functional structures that can enhance therapeutic or diagnostic performance. The approach can be considered analogous to the cellular process known as autophagy, where cellular components such as proteins and organelles are turned over for new cell formation. Silicon nanostructures possess two chemical features that allow for inorganic autophagy—the reducing ability of elemental silicon and the low solubility of silicate byproducts. Although bulk silicon is generally too stable to exhibit any significant degradation in biological media, nanoscale silicon can be oxidized and dissolved. For example, in the presence of silver ion, metallic silver can be formed by electroless deposition within a porous Si nanoparticle, generating composite nanoparticles with dimensions and plasmonic properties controlled by the porous nanostructure. These materials can be used as imaging probes or as antibacterial agents in vivo. Alternatively, dissolution of Si generates orthosilicic acid, which precipitates in the presence of calcium ion to form a structural shell of calcium silicate that can be used to trap oligonucleotides for gene therapeutics. This presentation will discuss the chemistry and photochemistry of nanostructured porous silicon, with emphasis on the self-destruction and reconstruction processes that can be harnessed for various in vitro and in vivo imaging and drug delivery tasks.

Keywords: Bioanalytical, Biomedical, Biotechnology, Nanotechnology
Application Code: Biomedical
Methodology Code: Fluorescence/Luminescence
Metal-organic frameworks (MOFs) containing a high density of near-infrared emitting lanthanide cations (Ln3+) and organic sensitizers are potentially attractive materials for biological imaging applications, because they emit narrow, sharp, non-decaying signals. The MOF matrix provides a rigid scaffold for precisely organizing Ln3+ and sensitizers and protecting the Ln3+ from the environment and sources of non-radiative signal decay. Here, we present several MOF platforms for designing and constructing NIR-luminescent materials, and we report progress toward systematically optimizing their luminescence for biological applications.

Keywords: Bioanalytical, Nanotechnology, Near Infrared, UV-VIS Absorbance/Luminescence
Application Code: Bioanalytical
Methodology Code: Near Infrared
Traditional approaches to selective bioimaging rely largely on molecular recognition approaches where probe designs are based on developing a lock-and-key pair between receptor and analyte. We are creating and applying new chemical tools to study small-molecule signal/stress agents, whose transient nature poses a challenge for traditional recognition-based sensing strategies. This talk will present our latest results on developing new molecular imaging agents that utilize a reactivity-based approach to selective detection of reactive oxygen, sulfur, and carbon species and their use in live cell, tissue, and animal settings, where selectivity is imparted by chemical reactivity.

Keywords: Biomedical, Biosensors, Fluorescence, Sensors
Application Code: Bioanalytical
Methodology Code: Fluorescence/Luminescence
A discovery made in my laboratory resulted in a high-throughput analysis technology that had commercial applications. The technology was licensed and led to a startup company--Illumina. Illumina has become the market leader in next generation genomics tools and has transformed our understanding of biology and genetics. The company is now transforming the face of healthcare. The road from an academic discovery to the foundations of this transformative company will be described and general lessons will be provided.

Keywords: Biotechnology, Fiber Optics, Genomics, Nucleic Acids
Application Code: Genomics, Proteomics and Other 'Oomics
Methodology Code: New Method
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**Abstract Text**

Illumina was founded in the spring of 1998 and today has revenue in excess of $2.4B annually. The company’s evolution from a nascent start-up through its current leadership in DNA sequencing will be explored, with a look to the future where genomic information becomes ubiquitous.

**Keywords:** Genomics

**Application Code:** Other

**Methodology Code:** New Method
The traditional approach to screening for recessive genetic disorders has involved a limited number of diseases and has been mostly ethnicity based. Recent advances in molecular technology have allowed pan-ethnic screening for a large number of disorders to be performed in a cost effective manner. This presentation will describe the clinical benefits of expanded carrier screening and the impact of next generation sequencing on increasing the detection of at-risk carrier couples.
The ability to sequence millions of DNA fragments in a single test has opened the door to revolutionary, non-invasive Clinical applications based on analysis of cell-free DNA in human blood. The first such application to reach widespread clinical use has been the "liquid biopsy" of the placenta, commonly known as "non-invasive prenatal testing" (NIPT). The presentation will provide an overview of the biological and analytical principles behind NIPT and the rapid evolution of its Clinical applications.

**Keywords:** Bioinformatics

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

**Methodology Code:** New Method
The obstacles to developing a safe and effective cancer screening tests are numerous, including biological, technical, and clinical challenges. The development of new tools that directly measure the sin qua non of cancer (its somatic alterations) has created new possibilities for methods of cancer detection. In particular, low-cost next generation sequencing has enabled the sequencing of tumor-derived nucleic acids circulating in the blood. This non-invasive characterization of a tumor’s genome combined with new methods of processing complex data may enable sensitive and specific detection of lethal cancers when they are still curable through surgery.
The Current State of the Art in (U)HPLC Columns
Possibilities and Limitations of State-of-the-Art UHPLC Columns and Systems

Both the introduction of ultra-high pressure (UHPLC) instrumentation and a new generation of superficially porous particles with a thick porous layer, have resulted in large leaps in separation performance over the last decade. In this lecture, it will be illustrated how the increase in operating pressure and improvement of column performance leads to faster or better separations (or both), using the so-called kinetic gain factors that allow to calculate the maximal performance gain. With higher operating pressures, it became possible to use smaller particles, yielding lower plate heights and higher optimal velocities. The particles are however packed in shorter columns to avoid too large pressure drops and with a narrower inner diameter to minimize solvent consumption and alleviate possible negative effects due to viscous heating.

The overall dispersion contribution of the chromatographic column is however proportional to the square of its volume. When e.g. switching from a classical 25cm long 4.6mm ID column packed with 5µm particles, to a 1.7µm particle column only 10cm long and with an 2.1mmn ID, the volume is decreased 12-fold and its resulting peak variance 144-fold. As a result, the overall band spreading that occurs in the chromatographic system will be much more affected by instrumental contributions, such as dispersion in tubing, preheaters, detector, injector etc. The challenge to obtain the intrinsic performance of state-of-the-art UHPLC columns therefore lies mostly on the instrument side as the most efficient columns today are ‘too good’ for the instrumentation available. The different contributions to this column dispersion will be discussed and possible methods for optimization and minimization of these effects explored. Using methodologies such as peak deconvolution and in-system dispersion measurements, the different extra-column effects can be quantified and compared with detailed numerical simulations of the flow behavior in the chromatographic system.

Keywords: Chromatography, HPLC Columns, Liquid Chromatography, Separation Sciences
Application Code: General Interest
Methodology Code: Liquid Chromatography
The separation power of UHPLC sorbents has been studied extensively using a variety of approaches including Van Deemter, Poppe and Kinetic plots. In the majority of these studies, corrections were made for the instrument contribution to band dispersion. While the conclusions from such studies are useful for hypothetical comparisons, an instrument with zero dispersion does not exist; therefore, these studies ignore an important factor that must be considered by practicing chromatographers. Additionally, various strategies to reduce instrument dispersion have been used such as different detector and injector designs as well as changing the diameter of connecting tubing. While reducing instrument dispersion is important, the cost of such optimization must also be considered, such as its impact on available instrument power.

In this work, the kinetic plot approach was used without correcting for instrument dispersion to allow more realistic modeling of the performance that can be achieved with currently available UHPLC systems. More importantly, practical limits were imposed on analysis time, column length, flow rate and column maximum operating pressure to focus results on the optimum particle morphology and size for real-world separation challenges. Results were further restricted to combinations of column formats (length and diameter) and operational ranges (flow rate and pressure) compatible with currently available columns and UHPLC systems. Various approaches to reducing instrument dispersion were evaluated against their costs and the impact of instrument dispersion was used to further refine the choice of column format.
Over the past decade there has been a concerted effort to increase separation efficiency in high performance liquid chromatography (HPLC). The benefits of increasing efficiency include faster analysis times and enhanced resolution. The trend began with the development of smaller particles (generally termed sub-2µm) and the instrumentation advances required to handle the resultant high-pressure requirements, establishing the age of ultra-high performance liquid chromatography (UHPLC). UHPLC using sub-2µm particles has gained widespread use in many industries; however, the financial burden of purchasing new instrumentation has hindered adoption by many potential users. The modern introduction of superficially porous particles (SPP) in 2006 provided a means of attaining high separation efficiencies with less backpressure burden. The lower backpressure afforded by the SPP particle architecture has allowed users of both traditional HPLC systems and UHPLC alike to realize high efficiency separations. Columns based on SPP have become the “gold standard” in many industries and continues to grow in others. A recent trend has been the application of SPP stationary phases outside of the traditional small-molecule reversed-phase arena. In this presentation we will discuss this new trend and explore the use of SPP columns in various chromatographic modes and specialized applications.
Superficially porous particle (SPP) technology has been rapidly adopted by chromatographers in the past several years due to its convincing performance advantage over totally porous particles (TPP) such as High efficiency and lower back pressure. Columns using SPPs are currently available in a wide variety of particle sizes, pore sizes and stationary phase chemistries to meet most analysts’ needs. This work will present an overview of current status of SPPs, syntheses of SPPs, and more recent development of expanding family of SPPs with different particle sizes and pore sizes for small molecule and large biomolecule separations, as well as future direction of SPPs. In addition, it will include an innovative hybridized superficially porous particle that is designed to resist degradation in high pH mobile phases. By using this high pH stable SPP column, one can run separation in low, neutral and high pHs as a way to gain different selectivity.
Low-density fluid chromatography (LDFC) is an extremely attractive and alternative separation technique because it may enable the analysts to solve complex mixtures containing both volatile compounds (usually analyzed separately by GC) and high-molecular-weight compounds (typically eluted by LC). The most commonly used low-density fluid is carbon dioxide: its viscosity is one order of magnitude smaller than that of water enabling faster and more efficient analyses than in LC; unlike gaseous mobile phase, its solubility parameter is easily tunable by adjusting temperature and pressure allowing the separation of various classes of compounds [1].

The challenge of LDFC used for the separation of volatile compounds is the severe cooling of the mobile phase as it decompresses along the column. This causes serious peak deformation with 2.1 to 4.6 mm i.d. columns. One effective solution to this resolution problem is to insulate thermally the chromatographic column in a 3 cm i.d. vacuum chamber at pressures smaller than 10-4 Torr.

In this presentation, it is demonstrated experimentally that LDFC (carbon dioxide) combined with high-vacuum technology (turbomolecular pump) can be successful to completely solve in a single run volatile compounds (flagrance, terpenes, n-alkanes from C5 to C20) present in complex mixtures containing heavy compounds (gasoline, plant extracts, petroleum products). A single 3.0 mm x 150 mm 2 [micro]m silica-C18 column, carbon dioxide (T>90oC and 1500 psi), and flame ionization detection are used. Volatiles are separated under isobaric conditions (1500 psi) while the high-molecular-weight compounds are separated by applying a linear pressure gradient at the column outlet (1500-3500 psi). The presented LDFC method enables the analysts to make the bridge between remote GC and LC analyses in a single, high-resolution, and fast chromatographic run.

A Symphony of Neurochemical Tools

Flexible Microelectrode Arrays for Monitoring and Manipulating Neuronal Dynamics

Electrical stimulation of brain tissue to induce or suppress neuronal activity is an important therapeutic application of implantable sensor technologies. Understanding why a specific stimulation is successful and what causes changes in the brain’s response to the same electrical stimulation over time is a key step to improving brain stimulation based therapies. This understanding requires the development of specialized microelectrode arrays (MEAs) and companion electronics that can simultaneously simulate and record 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. Iridium oxide electrodes on this probe can be used to electrically stimulate tissue, record unit activity or record pH changes. Integration of amperometric electrochemical sensors onto this same probe creates the opportunity to monitor changes in the extracellular concentrations of chemical messengers like glutamate but requires specialized plating and enzyme deposition techniques to fabricate sensors with both the high sensitivity and spatial resolution required on the MEA. These probes also include integrated reference and ground electrodes to minimize electrical noise and tissue damage during experiments. Use of these probes requires specialized methodology and equipment for both surgical implantation and later for electrical isolation of the two recording types and electrical stimulations. Finally, using these devices we are able to examine the dynamics of cell firing and glutamate release to electrical stimulations.

Keywords: Biosensors
Application Code: Neurochemistry
Methodology Code: Electrochemistry
Post-chemotherapy cognitive impairment, known also as “chemobrain”, is a medical complication of chemotherapy treatment that is characterized by a general decline in cognition affecting visual and verbal memory, attention, complex problem solving skills, and motor function. Developing an understanding of chemobrain is becoming more important as the survival rates of cancers continue to increase. Here, we used fast-scan cyclic voltammetry at carbon-fiber microelectrodes to measure the release and uptake of dopamine (DA), a CNS neurotransmitter that is involved in cognitive and motor processes in the brain striatum, in chemotherapy-treated rats. Male Wistar rats received IV injections of saline vehicle and Carboplatin (5 and 20 mg/kg), a chemotherapeutic agent that is associated with chemobrain in humans. Brain slices were acutely harvested from these rats and dopamine release and uptake was measured in four separate quadrants of the striatum. Reserve pool DA was measured by pretreatment with alpha-methyl-para-tyrosine (50 μM), which inhibits dopamine synthesis, followed by treatment with amphetamine (20 μM), which induces dopamine efflux from terminals. Modeling the stimulated dopamine release plots revealed an impairment of dopamine release per stimulus pulse (80% of saline control at 5 mg/kg and 58% at 20 mg/kg) after 4 weeks of carboplatin treatment. Moreover, Vmax, the maximum uptake rate of dopamine, was also decreased (55% of saline control at 5 mg/kg and 57% at 20 mg/kg). Nevertheless, overall DA content, measured in striatal brain lysates by high performance liquid chromatography, and reserve pool DA, measured by FSCV after pharmacological manipulation, did not significantly change. Measurements of spatial learning discrimination were taken throughout the treatment period and carboplatin was found to alter cognition.

Keywords: Electrochemistry, Microelectrode, Neurochemistry, Voltammetry
Application Code: Neurochemistry
Methodology Code: Electrochemistry
Insulin Increases Striatal Cholinergic Interneuron Excitability and Enhances Dopamine Release via nAChRs: Implications for Food Reward

Insulin can act on brain reward pathways to regulate feeding behavior. We used a variety of neurochemical tools to probe mechanisms by which this occurs. First, fast-scan cyclic voltammetry was used to monitor insulin-induced changes in evoked extracellular dopamine concentration ([DA]o) in striatal slices. Changes in DA transporter kinetics, were evaluated by fitting the initial falling phase of evoked [DA]o curves to the Michaelis-Menten equation to extract Vmax (maximal uptake rate). Insulin enhanced evoked [DA]o despite increasing Vmax. Importantly, insulin’s effect on DA release and uptake was prevented by HNMPA, an insulin receptor (InsR) inhibitor and by LY-294002, a PI3 kinase inhibitor, but not picropodophyllin (PPP) which blocks insulin-like growth factor 1 receptors. Second, immunohistochemistry revealed that although DA axons possess InsRs, abundant InsR expression was on cholinergic interneurons (ChIs) identified by co-staining for choline acetyltransferase (ChAT), the primary acetylcholine (ACh) synthesizing enzyme. Third, using whole-cell current-clamp recording in ChIs we found that insulin increased ChI excitability by attenuating spike frequency adaptation and increasing spike number for each step of a series of 3 s depolarizing current pulses. This effect was also prevented by HNMPA but not PPP. Because ChIs potently regulate DA release via nicotinic ACh receptors (nAChRs) on DA axons, we tested their role in insulin’s effects. Indeed, insulin failed to enhance evoked [DA]o when nAChRs were blocked, or evoked [DA]o and ChI excitability in mice lacking forebrain ChAT. Last, in behavioral studies intact insulin levels in the ventral striatum were necessary for acquisition of preference for the flavor of a paired glucose solution. Thus, InsR expression on striatal ChIs, increased ChI excitability with acute insulin, and nAChR-dependence of insulin-enhanced DA release implicate ChIs as insulin targets in the brain that perhaps influence food reward.

Keywords: Electrochemistry, Microelectrode, Voltammetry
Application Code: Neurochemistry
Methodology Code: Electrochemistry
Brain-computer interfaces (BCIs) have allowed control of prosthetic limbs in paralyzed patients. Unfortunately, the electrodes of the BCI that interface with the brain only function for a short period of time before the signal quality on these electrodes becomes substantially diminished. To truly realize the potential of BCIs, it is imperative to have electrodes that function chronically. In order to elucidate the physiological determinants of a chronically functional neural interface, we studied the role of the blood–brain barrier (BBB) in electrode function, because it is a key mediator of neuronal hemostasis. We also studied the relationship between electrode design, defined by shape, size and tethering; and long-term (chronic) stability of the neuron–electrode interface. We monitored the status of the BBB and the consequences of BBB breach on electrode function using non-invasive imaging, electrophysiology, genomic, and histological analyses. Rats implanted with commercially available intracortical electrodes demonstrated an inverse correlation between electrode performance and BBB breach over a period of 16 weeks. Genomic analysis showed that chronically functional electrodes elicit an enhanced wound healing response. Conversely, in poorly functioning electrodes, chronic BBB breach led to local accumulation of neurotoxic factors such as interleukins and tumor necrosis factor alpha, and an influx of pro-inflammatory myeloid cells, which negatively affect neuronal health. These findings were further verified in a subset of electrodes with graded electrophysiological performance. In this study, we determine the mechanistic link between intracortical electrode function and failure. Our results indicate that BBB status is a critical physiological determinant of intracortical electrode function and can inform rational electrode design and assessment, and biochemical intervention strategies to enhance the functional longevity of BCIs in the future.
Increasing the Speed and Sensitivity of Neurotransmitter Analysis by LC-MS

LC-MS can be utilized to simultaneously measure a wide range of neurotransmitters in dialysate samples. Many neurotransmitters are polar and are poorly retained on reversed phase LC. Derivatizing such analytes with benzoyl chloride can increase retention in reversed phase LC. Previous method development in our lab for neurotransmitter analysis by LC-MS was limited to 700 bar, which restricted the overall separation speed. A new generation of UHPLC pumps with a maximum pressure of 1500 bar is now available and was used to improve the throughput of dialysate analysis by LC-MS. However, at high flow rates and pressures, viscous friction caused the formation of thermal gradients within the LC column. These thermal gradients can lead to retention time shifts and losses in chromatographic efficiency. By utilizing thermal environment control integrated into a new instrument oven, the effects of thermal gradients arising from viscous friction can be better controlled. Here, we compared the impact of still-air and forced-air heating modes on mobile phase temperature increases and method repeatability. It was found that elution time RSD was less than 1.5% and peak area ratio RSD was no higher than 8% for a set of neurotransmitters even under conditions where the magnitude of the axial thermal gradient exceeded 14 K. The use of smaller column dimensions and lower volume connections to improve limits of detection and method sensitivity was also investigated.

Keywords: Liquid Chromatography, Liquid Chromatography/Mass Spectroscopy, Method Development, Neuroc
Application Code: Other
Methodology Code: Liquid Chromatography/Mass Spectrometry
A Symphony of Neurochemical Tools

New Tools for Rapid Clinical Neurochemical Monitoring

Traumatic brain injury (TBI) is the major cause of disability and morbidity of adults under the age of 35. After the irreversible primary damage occurred at impact, 40% of the TBI patients develop secondary brain damage in the following days. Spontaneous waves, termed spreading depolarizations (SD), propagate from the injury core to surrounding healthy tissue, almost completely depolarizing all neurons and astrocytes, leading to secondary brain injury and poor patient outcomes [1]. Recovery from this silencing phase relies heavily on the supply of metabolites to the tissue, as seen from a transient increase of ions and decrease of metabolites during an SD [2,3].

Our goal is to detect dynamic neurochemical changes with high time resolution using online clinical microdialysis. For this, we have built a bedside microfluidic monitoring system for real-time quantification of ions and metabolites [3]. To make the response faster, we use two strategies – improving the analytics and improving the tissue. Using retro-delivery of dexamethasone, an anti-inflammatory drug, the probe-tissue interface is better preserved, foreign body response minimized, and we investigate the enhancement in microdialysis output. To maintain and resolve this improved tissue response, we couple it with segmented flow microfluidics. Upon leaving the probe, the dialysate stream is immediately broken up into discrete droplets suspended in carrier oil. This eliminates Taylor dispersion hence efficiently capturing the dynamic concentration characteristics of the sample tissue.


Keywords: Biomedical, Electrochemistry, Lab-on-a-Chip/Microfluidics, Neurochemistry
Application Code: Neurochemistry
Methodology Code: Microfluidics/Lab-on-a-Chip
The neurochemical transmitter dopamine (DA) is implicated in a number of diseases states, including Parkinson’s disease, schizophrenia and drug abuse. DA terminal fields in the dorsal striatum and core region of the nucleus accumbens in the rat brain are organized as patchworks of fast and slow kinetic domains. The striatum is composed of a mosaic of spatial compartments known as the striosomes (patches) and the matrix. Extensive literature exists on the spatial organization of the patch and matrix compartments and their functions. However, little is known about these compartments as they relate to fast and slow kinetic DA domains observed by fast scan cyclic voltammetry.

In our study, we combined high spatial resolution voltammetric mapping with detailed immunohistochemical analysis of these architectural compartments (patch and matrix) by labeling for the µ-opiate receptor and using fluorescence microscopy. Our findings demonstrated a direct correlation between patch compartments with fast domain DA kinetics and matrix compartments to slow domain DA kinetics. We also investigated the kinetic domains in two very distinct sub-regions in the striatum. The lateral striatum as opposed to the medial striatum is mainly governed by fast kinetic DA domains. Upon treatment with the uptake inhibitor bupropion, recording sites exhibited changes in subsecond plasticity of release which was mathematically consistent with DA exocytosis literature. These finding are highly relevant as they may hold key promise in unraveling the fast and slow kinetic DA domains and their physiological significance.
Analysis using portable gas chromatography-mass spectrometry (GC-MS) allows for the conclusive identification of toxic chemical agents at the scene. This capability is important to law enforcement organizations and the military, providing rapid analysis in near-real time. Data generated may be used for many purposes including establishment of scene awareness, investigation and adjudication of crimes and criminal activity, support of counterterrorism efforts, and personal and public safety in both civilian and military arenas. The United States military uses portable GC-MS to achieve a number of goals. Protection is one of the primary reasons these systems are deployed, empowering users to make tactical decisions based upon results from data collected. In addition, these systems are used to identify dangerous chemical agents, even when masked in high concentrations of interferents. This capability is especially important considering the current risks chemical threats from terrorist organizations pose to both the military and the public at large. This presentation will describe the role portable GC-MS plays for military users, specifically describing the advantages and limitations that have been recognized as the use of these systems by military organizations continues to expand.

Keywords: Forensics, Gas Chromatography/Mass Spectrometry, Portable Instruments, SPME
Application Code: Homeland Security/Forensics
Methodology Code: Gas Chromatography/Mass Spectrometry
Near Infrared (NIR) spectrometers have been used in agriculture for over 50 years to measure the composition and properties of commodities and food products. There are many very good NIR spectrometers all designed to acquire the best spectrum within specified design parameters. This almost always entails some degree of sample preparation, drying, grinding, dissolving or orienting. Unfortunately this always changes the sample in some subtle and some not so subtle ways. To produce the most accurate description of the sample the spectrum should be obtained at the sample’s source location and without any chance for modification. This requires a different class of NIR equipment. The new group of handheld instruments are ideal for their portability and ease of in the field. The problem with agricultural samples is that they are generally large and inhomogeneous and it is incumbent on the analyst to develop a sampling scheme to adequately measure the sample. This requires that a number of spectra must be acquired at numerous locations on the sample to have a representative average spectrum of the material to be analyzed. We report the use of a high performance NIR spectrometer for field, processing plant and laboratory use. The spot size is generally around 30 cm which is much larger than the 1-2 mm of the handheld instruments and the 2 cm of most laboratory instruments. The instrument is larger than the handheld, but is transportable. Examples of use in processing plants and field use will be discussed.
Field Spectroscopic Analysis: Environmental, Pharmaceutical and Security Applications

Deep-Ultraviolet (DUV) Raman Spectroscopy for the Standoff Detection of Threat Materials

Alakai Defense Systems has created a family of deep-ultraviolet (DUV) Raman explosive detection systems operating at ranges from a few inches to hundreds of meters. These systems detect a variety of materials, from explosives and their respective precursors to chemicals used in the production of CWAs. This presentation will provide an overview of the different system capabilities and discuss some key parameters for detecting materials of specific application interest. We will also address the impact of eye-safety concerns on standoff Raman detection.

Keywords: Forensics, Portable Instruments, Raman Spectroscopy
Application Code: Homeland Security/Forensics
Methodology Code: Portable Instruments
# Field Spectroscopic Analysis: Environmental, Pharmaceutical and Security Applications

## Use of a Field Portable GC/MS with Solid Phase Microextraction and Needle Trap Sampling for VOC and SVOC Analysis

A simple field-portable device for quick and quantitative sampling is described. In this study, we report on the use of field-portable gas chromatography–mass spectrometry (GC–MS) with solid-phase microextraction, thermal desorption, and needle trap sampling techniques to provide a fast response for in-field analysis of volatile and semivolatile organic compounds (SVOCs) in a wide variety of environmental and industrial-type samples including potable waters, tea, plants, and road gravel. A needle trap is ideally suited for use with a field portable High Speed High Resolution GC/MS. A needle trap is a micro version of the more common thermal desorption traps used in laboratory systems. We demonstrate that this field-portable approach can provide the required sensitivity and selectivity for the effective analysis of VOCs and SVOCs with varying boiling points such as polycyclic aromatic hydrocarbons (PAHs), pesticides, phenolic compounds, and phthalate esters in a number of different field-based samples, in less than 5 min. Examples are given for volatile and semi-volatile analytes in air with detection limits in the low ppb range.

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

**Application Code:** Environmental

**Methodology Code:** Portable Instruments
Field Spectroscopic Analysis: Environmental, Pharmaceutical and Security Applications

A Novel FTIR / GC-FTIR Analyzer for Environmental Monitoring

Current environmental monitoring regulations for atmospheric emissions require the ability to qualify and quantify many different compounds emitted at levels from % to ppbv. Companies testing their emissions prefer to have real-time or near real-time results so they can improve their environmental processes and avoid significant non-compliance fines. Due to the many different types of chemicals that can be emitted, oxides (NO, NO\(_2\), CO, CO\(_2\), SO\(_2\)), inorganic acids (HCl, HF), organic acids (acetic acid), hydrides (AsH\(_3\), PH\(_3\)), VOCs, SVOCs, and H\(_2\)O, it is difficult if not impossible to measure for all these compounds with a single analyzer in real-time or even near real-time due to interferences and instrumental sensitivity.

A novel technology has been developed that couples an FTIR gas analyzer (capable of real-time simultaneous monitoring for many compounds from % to high ppbv) with a GC-FTIR that can measure 100s of compounds in the ppmv to sub-ppbv range in near real-time. These two coupled analyzers now allow for the monitoring of nearly any process or environmental gas stream. By using an FTIR as the detector for both measurements, 9 orders of dynamic range can be obtained while routine calibrations and full compound separation are not required. Along with this novel instrumental technology, state-of-the-art analytical software allows for the observation and quantification of analytes that are 10,000 times lower than a spectrally interfering compound. Data demonstrating the operation of the FTIR / GC-FTIR and its software on environmental samples will be presented.

Keywords: Environmental/Air, FTIR, Gas Chromatography, GC Detectors
Application Code: Environmental
Methodology Code: Vibrational Spectroscopy
The drive of portable instrumentation is to bring detection to areas and those that would otherwise not have it without a laboratory setting. This bar has continually been pushed out into the real-world through the evolution of electronics and the conversion of laboratory instrumentation to the field through shrinking and simplifying. Detectachem has created a product called MobileDetect for this in the field of Drug detection. Given there are advanced algorithms and other specific hardware needed for many of these portable instruments, the current push is to continue to provide these capabilities to those that do not desire or cannot afford a specific piece of hardware. Introduce the age of the Smartphone with high resolution camera capability. Detectachem has taken this a step further with the capability to detect trace amounts of drugs with the partnering of colorimetric chemicals delivered in a calculated approach. Using a smartphone and the Detectachem “Smart Pouch”, the user and common person can utilize this platform to reliably and accurately detect various amounts and types of illicit drugs. This capability pushes detection out to Law Enforcement, schools, state and local agencies and even parents. Merely taking a picture is not all that is needed as the detection environment requires specific manipulation and accommodation to different lighting conditions, variance in imaging systems and other variables that are unable to be ultimately controlled by the user. The development of Mobile Detect will allow users to perform a simple yet advanced detection of these illicit drugs through unrealized field spectroscopy. It is unrealized because all the processing and algorithms including controlling the user interaction built into the smartphone Application ensuring correct processes are followed. The field capability with trace detection is an industry-leading addition in capability to the users and a cutting edge application of Automated Colorimetrics.

Keywords: Detection, Drugs, Portable Instruments
Application Code: Drug Discovery
Methodology Code: Portable Instruments
Field Spectroscopic Analysis: Environmental, Pharmaceutical and Security Applications

Portable Forensic Mass Spectrometry

Instrumental methods of chemical analysis provide objective and largely irrefutable circumstantial evidence in the support of criminal prosecutions. Chemical measurements can also be archived for future validation or cross-examination by third-party expert witnesses, and they are widely accepted as amongst the most reliable of forensic techniques. It is for these reasons that chemical measurements received almost no criticism in the highly critical 2009 National Academy of Sciences (NAS) report on the Strengthening Forensic Science in the United States: A Path Forward. More recently, several recent advancements have been made in different areas of instrumental analysis that has resulted in a shift towards on-site and in situ chemical measurements. The forensic community therefore has some important decisions to make regarding the future of portable instrumentation and its application in forensic casework.

The first part of this presentation will evaluate the pros and cons on on-site or in situ measurements in forensic casework. This evaluation will include a return-on-investment analysis that attempts to incorporate the criminal justice system as a whole, and not just the crime laboratory system. The second part of the talk will be more focused on instrumentation development, more specifically, the development of a new type of high-pressure mass filter that is innately suited for miniaturization. The Loeb-Eiber mass filter is based on the preliminary work of Hans Eiber in 1963 in which he demonstrated the ability to mass separate different molecular species of oxygen at around 1 Torr. Our most recent work in this area uses microfabricated chips with mass filter electrodes on the order of 25 \( \mu \text{m} \) in diameter, and that operate with rf waveforms on the order of 0-20 Vpp. The ability to operate at high pressures (~ 1 Torr) and low voltages (0-20 Vpp) makes the Loeb-Eiber filter a promising candidate for development as a miniature, portable instrument.
Until recently NIR spectrometers could generally be separated into two broad classes: high performance benchtop units designed for laboratory use and lower performance handheld, or highly portable, spectrometers designed for field use. In this context “lower performance” could mean limited wavelength (or wavenumber) range, limited spectral resolution, limited signal-to-noise ratio, limited instrument stability over time (usually wavelength stability), limited instrument to instrument reproducibility, or some combination of these factors. In many cases these compromises in performance do not preclude the portable instrument from fulfilling the requirements of a given application, but this is not always the case.

In this talk we will discuss the design of a series of high performance Fourier transform NIR spectrometers that are rugged, small, and light enough to be portable, field deployable, systems. These new spectrometers were designed to fill the gap in the current instrumentation described above. We will present data showing the relative performance of these new instruments and leading brand benchtop instruments, as well as calibration transfer studies showing how both qualitative and quantitative models may be moved very easily from an existing benchtop FT spectrometer to the new portable FT spectrometer. We will also present a number of example applications that benefit from this new instrumentation.

Keywords: Instrumentation, Near Infrared, Portable Instruments, Spectroscopy
Application Code: Pharmaceutical
Methodology Code: Near Infrared
A simple and sensitive method for simultaneous microextraction and determination of heavy metals using pencil lead was investigated. In this method, polypyrrole–carbon nanotube composites doped with 1, 10-phenanthroline were electrodeposited on pencil lead and used as the fiber for solid phase microextraction (SPME). This SPME method was then combined with inductively coupled plasma mass spectrometry (ICP-MS) for trace determination of heavy metals. The effect of the amount of carbon nanotube and 1, 10-phenanthroline, and electrodeposition time for electropolymerization step were optimized. The parameters influencing microextraction efficiency such as pH, microextraction time, and desorption time were also optimized. The effect of inorganic interferences on the determination of the heavy metals was also examined. The results showed selectivity of the modified SPME fiber toward cadmium, cobalt, lead, nickel, silver, and zinc in the presence of more than forty metal ions. Preliminary results demonstrated that enrichment factors for these metals were greater than fifty. Finally, these heavy metals were determined in natural waters since the trace concentration of these metal ions are usually less than the detection limit for ICP-MS.
The exposure and dose of nanoparticle (NP) contaminants to cells in either human or environmental health is a subject of great interest and debate. In the medical profession it is important to know how much of a NP containing drug is actually entering the target cells, and in the environment it is important to know whether NPs from consumer products are affecting the ecosystem but interacting with algae or bacteria and if these can then be trophically transferred through the food chain. It is generally accepted that desirable metrics for measuring exposure and dose in this case would be particles per mL (part. / mL) and concentration per cell (conc. / cell) respectively. However because of instrument limitations these have both been measured using ionic mass concentration which means there is some vital missing information.

Here we exposed various strains of freshwater algae to Au NPs and Au ions. We show that single cell ICP-MS (SC-ICP-MS) was able to measure the number of NPs in the suspension media (exposure), the number of cells containing metal (% uptake) and finally the amount of metal within those cells (dose) in various different species of freshwater algae cells. We show that although the exposures are kept constant (ionic concentration of 1 ppb, equating to a NP concentration of around 500,000 part. / mL) the dose and response is highly dependent on the algae strain.
Several hexavalent chromium standards in soil, certified in the past decade, either have very high concentrations of chromium or active matrices that render them unsuitable for validating natural soil and natural background studies. NIST 2701, for example, is made of chromium processing ore residue (COPR) industrial waste material from New Jersey. It contains approximately four percent total chromium, with an unnaturally high Cr(VI) fraction (552.1 mg/kg). SRM-2701 also includes an active matrix that dominates the chemistry of the standard and causes significant Cr(III)/Cr(VI) species shift and biases during extraction. Presently, no low-background level standard containing an inert matrix is available for the appropriate validation of low level Cr(VI) in native or uncontaminated soil and low level risk assessment. An international collaboration is under way to produce a more appropriate series of low level soil certified standard reference materials for Cr(VI) analysis. Multiple laboratories in different locations are involved in a collaborative effort to certify two new reference materials at ng/g levels. These were prepared by Sigma-Aldrich in larger batches and were distributed among the participating laboratories for collaborative certification. The methods used for certification are two EPA RCRA methods: alkaline extraction by EPA method 3060A and speciated isotope dilution mass spectrometry by EPA method 6800 (Update V, 2015). The international collaboration results and the analytical methods used in the preparation of the materials and preliminary certification, including means and confidence limits, will be presented along with some discussion of the Eh and Ph phase diagram stability of the material.
Developments in microcontroller and consumer battery technologies have facilitated the development of several advanced portable instruments. Over the past year, we have developed a hand held portable potentiostat operating on the Arduino microcontroller platform. This instrument has several key features making it ideal for in-the-field measurement and analysis. The final assembled instrument is highly portable, with a footprint of just a few inches and a battery life of over 15 days, assuming several hours of daily use. It has a sweep rate range of 0-400 mV/s, and is currently programmed to perform several electrochemical experiments. The instrument is capable of autonomous on-the-fly gain control and self-calibration, and has a linear range spanning six orders of magnitude. This feature allows the user to conduct electrochemical experiments on unknown systems without prior knowledge of the expected current output. The instrument is capable of detecting and storing background subtracted peak currents, allowing autonomous analysis of unknown analytes when coupled to the appropriate experimental controls. Finally, the instrument is capable of storing thousands of experiments in internal memory, and costs just $100 to produce. These features allow the device to make measurements of several metals in ground water, including copper, lead, and cadmium, in the low parts per billion range. We quantified these metals directly and autonomously using our device, independent of user input, and without the need for a laptop or personal computer.
[**In-situ**] trace metal analysis is critically important for environmental chemical analysis since the process of sampling disrupts the natural state of environmental analytes and changes speciation (which primarily controls toxicity and mobility). Electrochemistry shows great promise for [**In-situ**] analysis because electrodes are portable and can be immersed directly into systems of interest. However, sensor selectivity and stability are ongoing issues for electrochemical trace metal sensing.

We recently created a highly selective and stable Cu\(^{2+}\) sensor procedure by covalently modifying the surface of carbon fiber microelectrodes (CFMs) with a Cu\(^{2+}\) ionophore. This work investigates the effects of pH, ionic strength and solution composition on the sensor’s response and establishes the thermodynamic equilibrium constant for Cu adsorption onto CFMs. A variety of untreated, natural samples are characterized with this novel sensor. Finally, we introduce our design for an on-site trace metal sensor, a prototype for a Hg-free voltammetric tool with excellent temporal and spatial resolution.

**Keywords:** Chemically Modified Electrodes, Environmental/Water, Portable Instruments, Trace Analysis

**Application Code:** Environmental

**Methodology Code:** Electrochemistry
Toxic heavy metals such as lead (Pb), cadmium (Cd), and mercury (Hg) cause serious health complications and ingestion of these toxins through contaminated drinking water, even at trace levels, has become a prominent issue. Chronic exposure to toxic metals such as Pb, Cd, and Hg is carcinogenic while causing other problems like kidney failure, severe neurotoxicity, and IQ loss. These problems are only magnified in children as several stages of bodily development can be severely hindered. Electroanalytical methods are an attractive technique for trace detection of heavy metals due to low cost of experimental tools, low limits of detection, multi-element analysis capability, and the possibility to package them into sensing devices. Specifically, boron-doped diamond (BDD) is a rugged, yet sensitive electrode material with significant potential in electrochemical sensing. Using square-wave stripping voltammetry (SWSV), we have developed BDD micro-electrode arrays (MEAs) as well as macro-electrode disks, achieving detection limits as low as 160 parts-per-trillion (ppt) for Pb with a deposition time of just 2 minutes. This is nearly 100x below the 15 ppb maximum contaminant level (MCL) in drinking water set by the Environmental Protection Agency (EPA). MEAs of various diameter and spacing were investigated to find the optimum geometry for both single and multi-element detection of Pb, Cd, Hg, and Mn where results were compared with those obtained at various macro-electrode sizes. The applicability of each electrode for the determination of toxic heavy metal ions in drinking and environmental water samples was studied as well.

Keywords: Lead, Stripping Analysis, Trace Analysis, Water
Application Code: Environmental
Methodology Code: Electrochemistry
Heavy metal ions present in sea habitats are found bio-accumulated in tissues of several living organisms, e.g. oysters and mussels. Chemical groups (such as amide, amine, hydroxyl, carboxylic, imidazole, sulfate, phosphate, thiol and sulfhydryl) present in cells of related tissues provide the chemical basis to bind these metals. In this context, the sulfur atom present in some key amino acids such as cysteine and cystine, has the ability to complex most of those pollutants. This characteristics can be exploited as basic factor of oysters and mussels for their role as sea pollutant biomarkers. Few tools are known to study the role of sulfur in biological systems. Among them, X-ray absorption near edge structure (XANES) spectroscopy offers a unique non-destructing method to determine sulfur chemical speciation [1].

This study reports the sulfur speciation of characterized Atlantic oysters and Mediterranean mussels samples using X-ray absorption near edge structure (XANES) spectroscopy. This noninvasive method provides direct information and evidences of organic and inorganic forms, allowing to identify the sulfur complexes. The obtained spectra have been analyzed and compared with synthesized Zinc metallothioneins (Zn-MTs) and other sulfur references found in literature [2]. Gaussian combination (GC) and linear combination (LC) methods have been employed to determine sulfur speciation. Estimation of Zn-MTs content has been provided, also including both organic and inorganic forms of sulfur in target samples. The obtained results correlate well with heavy metals content in target samples, observing oysters or mussels to be appropriate sea biomarkers for heavy metals pollution.

Refs:

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Keywords: Atomic Spectroscopy, Environmental/Biological Samples, Speciation, Sulfur
Application Code: Environmental
Methodology Code: High-Energy Spectroscopy
Environmental Analysis of Pesticides, Hydrocarbons, and Other Organics

The Analysis of Polar Ionic Pesticides by Ion-exchange Chromatography Tandem Mass Spectrometry: The Possible Solution to a Longstanding Problematic Analysis?

Polar ionic pesticides, such as glyphosate, perchlorate, chlorate and the like, often occur as residues in food, but are not always included in pesticide monitoring programs, simply because they are not ‘amenable’ to generic multi-residue methods. The introduction of the Quick Polar Pesticides (QuPPe) Method by the European Reference Laboratory for single residue methods (EURL-SRM) has enabled more laboratories to conduct analysis for at least some of the polar pesticides. Still, the absence of a liquid partitioning step, or clean-up step, results in ‘dirty extracts’ containing high concentrations of matrix co-extractives. Thus, the separation and accurate quantification of analytes in QuPPe extracts is challenging. Analysts attempt to mitigate these issues by analyzing a single extract a number of times, using different chromatographic columns and conditions. These separation conditions are often less than ideal and the large amounts of co-extractives often contaminate the low capacity columns to cause variation in retention time and a decrease in the ruggedness of the method.

The application of high resolution ion-exchange chromatography with high capacity columns, coupled to a triple quadrupole mass spectrometer can overcome the issues experienced with other chromatographic techniques. Using the IC-MS/MS approach for direct analysis of QuPPe extracts, low limits of quantification (typically < 5 ng/g), and associated repeatability (typically < 20%) have been achieved for chlorate, perchlorate, glufosinate, N-acetyl glufosinate, 3-MPPA, glyphosate, AMPA, Fosetyl-Al, phosphonic acid, ethephon, HEPA and more, in a single analysis. Further details on separation, quantification and validation in various matrices will be presented.

Keywords: Ion Chromatography, Ion Exchange, Mass Spectrometry, Pesticides
Application Code: Environmental
Methodology Code: Liquid Chromatography/Mass Spectrometry
Environmental Analysis of Pesticides, Hydrocarbons, and Other Organics

On-Site, Thin-Film Microextraction for the Quantitation of Anthropogenic Pollutants in Surface Waters Using Portable GC-MS Instrumentation Validated by Comparison to Benchtop Methods

The advent of hand portal GC-MS instrumentation provides a unique opportunity for the immediate determination of contaminants in environmental systems. This expedience is of particular importance when such contamination may pose a threat to public safety such as with benzene, toluene, ethylbenzene and xylene (BTEX) markers associated with oil spills. However, a major limitation of these miniaturized detectors is the loss of instrument sensitivity resulting in limits of detection that are order(s) of magnitude higher than that of their benchtop counterparts.

It is therefore the goal of the proposed work to demonstrate a high sensitivity thin film microextraction (TFME) method to make up for the limited sensitivity of portable instrumentation. The orders of magnitude enhancement in extraction efficiency provided by TFME over classical solid phase microextraction (SPME) was shown to give similar on-site method limits of detection (MLOD’s) as what is achievable when a comparable SPME fibre was employed with a single quadrupole benchtop GC-MS instrument. These MLOD’s were shown to be 1 ppb or lower for most of the 20 multi-class pesticides spiked in either nanonpure or surface water collected from an Ontario river which are on-par with those values required by an accredited liquid-liquid extraction (LLE) EPA method. Finally, the proposed method was fully employed on-site and compared with benchtop methods for the quantitative determination of BTEX and any of the aforementioned 20 pesticides when found in the 4 riparian sampling location chosen in Southern Ontario, Canada. As such, this novel on-site TFME method was demonstrated to give similar limits of detection as what is achievable by benchtop instrumentation using classical sample preparation techniques with the added benefit of immediate results. Despite being new to the analytical community such on-site works may eventually prove to be pioneering in the field of on-site environmental analytical chemistry.

Keywords: Environmental Analysis, GC-MS, Pesticides, SPME

Application Code: Environmental

Methodology Code: Sampling and Sample Preparation
The goal of this study was to directly compare gas chromatography coupled to atmospheric pressure ionization tandem mass spectrometry (APGC-MS/MS), electron ionization tandem mass spectrometry (EI), and quadrupole time of flight mass spectrometry (Q-ToF) to high resolution mass spectrometry (HRMS) to demonstrate their validity and reliability in the analysis of halogenated dioxins and furans. The instruments compared to the Autospec GC-HRMS were: Water’s Xevo APGC TQ-S, Xevo APGC TQ-XS, and Xevo Q-Tof G2-XS as well as an Agilent 7000C GC-MS. Both the Water’s TQ-S and TQ-XS show single femtogram sensitivity. The instruments were compared using method detection limits (MDLs) from simulated extracted soil samples. The TQ-XS shows MDL levels lower than the GC-HRMS, while the TQ-S shows comparable MDLs to the GC-HRMS. The G2-XS and 7000C show slightly higher MDLs. Quantification of these soil samples were directly compared amongst the instruments with the TQ-XS performing slightly better than the GC-HRMS. Each of the other instruments performed as well as the GC-HRMS. The data presented in this paper is intended to validate these instruments in the analysis of halogenated dioxins and furans.

Keywords: Chromatography, Environmental Analysis, Gas Chromatography/Mass Spectrometry, Mass Spectrometry
Application Code: Environmental
Methodology Code: Gas Chromatography/Mass Spectrometry
US EPA method 625 is used to determine acidic, basic, and neutral semi-volatile organic compounds (SVOC) in municipal and industrial wastewater using GC-MS, in many cases for National Pollution Discharge Elimination System (NPDES) compliance. The most recent version of method 625, version 625.1 includes new language allowing the use of solid phase extraction. Additional requirements for laboratory validation with nine matrices optionally done at the vendor location or with a laboratory are required. This new language is also in several other new method versions, such as 608.3 and 624.1 to allow changes to the method and a re-validation procedure.

In this work we measured nine different wastewater-type matrices using the full suite of deuterated surrogates. Working with an accredited laboratory and the EPA Office of Science and Technology we were able to design a study and validate the method using an innovative solid phase extraction disk that requires one pass of the water rather than two passes with a pH adjustment to capture the full range of acid, base and neutral compounds. The performance of the various matrices in the study will be discussed and the advantages to an environmental laboratory will be outlined.

**Keywords:** Environmental Analysis, Environmental/Water, Gas Chromatography/Mass Spectrometry, Solid Phases

**Application Code:** Environmental

**Methodology Code:** Gas Chromatography/Mass Spectrometry
Environmental Analysis of Pesticides, Hydrocarbons, and Other Organics

The Characterization of Flowback Hydrocarbons Towards the Fingerprinting of Environmental Contamination Events

Hydraulic fracturing is an increasingly common technique for the extraction of natural gas entrapped in shale formations. This technique has been highly criticized due to the possibility of environmental contamination, underscoring the need for method development to enable point-source identification of environmental contamination events. Here, progress towards point-source identification and apportionment of environmental contamination events from unconventional gas development are presented. To date, our primary tool has been GCxGC coupled to mass spectrometry, a unique instrumental combination that allows for hydrocarbon fingerprinting. Through the analysis of a series of five injection fluids and five flowback samples from a single unconventional gas well in northeastern PA, it was revealed that hydrocarbon speciation remains constant during the flowback period. Additionally, analysis of flowback from four nearby wells by GCxGC coupled to a high-resolution time-of-flight mass spectrometer indicated a unique hydrocarbon pattern in each case. These findings demonstrate the potential of utilizing hydrocarbon signatures in flowback fluids towards the fingerprinting of environmental contamination that may occur as a consequence of unconventional gas development.

Keywords: Environmental, Gas Chromatography/Mass Spectrometry, Geochemistry, Hydrocarbons

Application Code: Environmental

Methodology Code: Gas Chromatography/Mass Spectrometry
Environmental Analysis of Pesticides, Hydrocarbons, and Other Organics
PAHs in Whole Water Using a New Method

Polycyclic aromatic hydrocarbons (PAHs) are found worldwide and emitted from a number of sources including fossil fuel, coal and shale oil derivatives, coke production and burning wood for home heating, generally arising from incomplete combustion. Surface water supplies, such as water in ponds may be used for recreational purposes or eventually end up in drinking water. Characterization of PAHs and their concentration is of interest in maintaining public health.

PAH measurement in water should be accurate, precise and sensitive to measure low concentrations. Method EN 16691 is a recently developed method that uses solid phase extraction to isolate organic compounds from 1 L of water using a divinylbenzene (DVB) solid phase extraction disk. PAHs are eluted from the disk with dichloromethane and dried to remove water before evaporation, solvent exchange into toluene and introduction into GC/MS. The method specifies the use of the whole water sample, ensuring that any analyte adsorbed on the particulate matter will be extracted along with the water sample. Disks are a particularly well suited SPE format for samples containing particulates because the increased surface area does not become clogged with particulate as easily as a cartridge format might, even for larger water samples, such as 1L. In addition, the particulates are rinsed with solvent at the same time as the bottle is rinsed in an automated system.

This work will demonstrate the performance of automated SPE for the extraction of PAHs from surface water sampled from a nearby pond and containing some particulate. An automated system with DVB disks, following the requirements of method EN 16691 is used.

Keywords: Environmental/Water, HPLC, PAH, Solid Phase Extraction
Application Code: Environmental
Methodology Code: Liquid Chromatography
The Miraflores Water Treatment Plant on the Panama Canal provides 40% of the drinking water for Panama City. Due to the level of traffic on the waterway there is a need to continuously monitor the VOC content of the water. When analyzing a large number of compounds by gas chromatography (GC), the separation of coeluting compounds can be an analytical challenge. In this application, fifty-eight volatile organic compounds (VOCs) were analyzed on two separate GC-MAID (Micro Argon Ionization Detector) instruments. The two GC systems were identical, except that one used a 100% PDMS column and the other used a 94% PDMS, 6% Cyanopropyl-phenyl column. The use of two different stationary phases resulted in a differing elution order on the two columns which ameliorated the coelution issue. The VOCs analyzed for this application were those of concern when managing the drinking water intake at the Panama Canal and were detected at levels down to 1 ppb. The instruments were integrated into the existing infrastructure and set up to continuously and autonomously monitor the water intake using purge and trap technology. Alarms are triggered when the concentration of any contaminant exceeds 2 ppb. It is important for water facilities to have access to on-going, reliable data about the concentration of VOCs present in drinking water and this application shows that it is possible to achieve this data using a GC system.

Keywords: Chromatography, Contamination, Environmental/Water, Gas Chromatography
Application Code: Environmental
Methodology Code: Gas Chromatography
Measurement of Formaldehyde Pollution in Ambient Air

Formaldehyde is commonly used chemical compound that exists in various forms and at room temperature, is a colorless, distinctive, strong and even pungent smelling, flammable and gaseous substance. Formaldehyde is an ozone precursor and classified as carcinogenic compound. It is highly injurious to health, but until today, it has been very difficult to measure. Levels above 0.1 ppm can cause acute health problems. It is commonly used chemical compound that is manufactured for use on an industrial scale. Formaldehyde is used e.g. for manufacturing of building materials, wood products and furniture. Formaldehyde in indoor air is thus caused by emissions from wood products. Formaldehyde in outdoor air again, is largely a result of photochemical processes and the use of biofuels.

Formaldehyde is monitored both in indoor and outdoor air and the regulation and recommendations for monitoring are constantly increasing. For ambient air, pollution monitoring is regulated and for indoor, measurement is done for occupational safety. That is, ambient air concentration limits have to be stricter as people are constantly exposed whereas in indoor air people are allowed for short time exposures if they are considered healthy persons.

We address the trace formaldehyde measurement need with widely tunable quantum cascade laser based photoacoustic detection [1,2]. Ultimately sub-ppb level detection limits are demonstrated which proves high suitability for the purpose of continuous Formaldehyde monitoring for ambient fugitive emissions as well as indoor air quality.


Keywords: Environmental/Air, Photoacoustic, Trace Analysis, Volatile Organic Compounds
Application Code: Environmental
Methodology Code: Molecular Spectroscopy
The variation among the wide range of formulations for coatings, polymers and materials continues to grow. Characterization of these vital products is very important for development, manufacture, processing, usage, and failure analysis. Pyrolysis-GC/MS is a very flexible technique which can be used sequentially in multiple modes to chemically characterize these types of samples. Strategies can include other analytical information in addition to PY-GC/MS data. This presentation uses a range of PY-GC/MS application examples to illustrate the utility of information obtained from different PY-GC/MS modes of use.

Analytical objectives for sample analysis may include identification and/or quantitative evaluations. Irrespective of the emphasis, the analysis by PY-GC/MS can be structured to reveal lower concentration volatile components, polymer backbone chemistry or other detailed compositional information about the mixture.

The best strategies for characterization begin with a Method Map for PY-GC/MS and this presentation will illustrate the range of modes of analysis and the results that are generated using these techniques.

Keywords: Characterization, Gas Chromatography/Mass Spectrometry, Polymers & Plastics, Pyrolysis
Subtle differences in polymer formulations are common, but they rarely influence the overall performance of the final product. However, there are instances where the product will ‘suddenly’ be more brittle, easily deformed or actually fail in routine use. When this occurs the search for differences begins. Detailed analyses of the “good” and “bad” polymer will often reveal differences in the additive package, the presence of unwanted contaminates or unexpected chemical linkages. A method map concept, that guides the analyst through the maze of analytical techniques used to characterize a polymeric material, will be discussed. The first step is to analyze a small aliquot (100µg) of the sample using Evolved Gas Analysis (EGA)-MS. The EGA thermogram provides a thermal profile of the sample. Average MS, extracted ion chromatograms and library search results are used to map the analytical sequence, which will provide valuable information about the constituents of the sample.

Pyrolysis (PY) and Heart-Cutting (HC) techniques will also be used to chemically characterize the differences between two rubber samples submitted for failure analysis. PY allows all components of the samples to be observed in one chromatogram (pyrogram). However these chromatograms are often too complex to observe subtle differences between samples. The HC technique allows multiple thermal zones of interest, identified from the initial EGA thermograms, to be chromatographed individually. HC chromatograms can isolate additives from the polymers for example.

In addition a novel approach of using a Pyrolyzer as the sample introduction technique followed by simultaneous data acquisition by multiple GC detectors (MS/FID/NPD) will be demonstrated.

Keywords: Characterization, Gas Chromatography/Mass Spectrometry, Polymers & Plastics, Pyrolysis
Application Code: Polymers and Plastics
Methodology Code: Gas Chromatography/Mass Spectrometry
Stir-bar sorptive extraction (SBSE) has been proven an effective and green extraction method for persistent organic pollutants (POPs) in aqueous samples such as human blood, drinking water, and wastewater. Traditionally, consistent recovery is required for accurate and precise measurements. Isotope dilution mass spectrometry (IDMS, EPA Method 6800) is a quantification method which eliminates the error from recovery and provides accurate and precise results without calibration curves. In this study, a method was developed to quantify POPs in different matrices using stir-bar sorptive extraction - thermal desorption - gas chromatography - mass spectrometry - isotope dilution mass spectrometry. This combination of extraction, desorption, separation, and quantification techniques enabled accurate and precise quantification of POPs in various matrices. This method was applied to aqueous samples such as human blood as well as solid samples including dietary supplements and other foods. IDMS was compared with calibration curves and was demonstrated to be a more accurate, precise, and efficient quantification method. The quadrupole mass analyzer was compared with a triple-quad mass analyzer (QqQ) which can provide a higher level of sensitivity. In trace analysis of POPs, improvement of sensitivity can be significant in leading to a lower detection and quantification limit. More than fifty POPs were investigated in dietary supplements, foods, and human blood. These POPs included pesticides and industrial chemicals which were recently found in children's blood and the environment. The method and results were provided to physicians to assist in the characterization of the human exposome.

In-vial extraction and coiled wire filament sampling/injection techniques were developed for rapid analysis of semi-volatile organic compounds (SVOCs) in water using a hand-portable GC-MS system. A TRIDION-9 GC-MS, equipped with a new resistively heated column bundle that can operate up to 320$^\circ$C at 120$^\circ$C/min ramp rate, allowed elution of SVOCs in less than 4 min with high resolution and rapid target analyte determination. The MS deconvolution and extracted-ion features of the TRIDION-9 allowed isolation of target analytes from complex sample matrices and improvement in detection limits while still retaining the MS identification information of total-ion-monitoring. Fieldable in-vial extraction utilized a small conical glass vial, often containing a polymer film coating. This technique required only 200-400 µL of solvent to accomplish target analyte extraction and concentration in less than 5 min with only 20-40 mL of sample. The coiled wire filament, a tiny deactivated stainless steel coil, enabled solvent-free sample injection from a constant sampling volume of up to 20 µL. The coiled wire filament eliminates sample discrimination that sometimes occurs with conventional syringe injection of high boiling point SVOCs, and it minimizes detection interference and system contamination caused by complex sample matrix residues. Eighteen polycyclic aromatic hydrocarbons including coronene, a very high boiling point compound; twenty biphenyl congeners; seventeen organochlorine pesticides; nineteen organophosphorus pesticides; nineteen phenolic compounds; ten pyrethroid-herbicides; six phthalate esters; n-alkanes from C10 to C40, as well as other SVOCs in water and waste water were determined at low ppb concentration levels in less than 10 min total analysis time.

Keywords: Environmental/Water, Gas Chromatography/Mass Spectrometry, Sample Introduction, Sample Prep
Application Code: Environmental
Methodology Code: Gas Chromatography/Mass Spectrometry
Software as a Service (SaaS) solutions have become increasingly popular for organizations across the industry spectrum. SaaS allows companies to deploy products without the need to invest in servers or ancillary software. This is beneficial for companies with limited IT resources, as the SaaS provider monitors and manages the software, including product updates, patches and backups. Additionally, SaaS solutions allow users to access the product from anywhere, via a secure platform.

There are several options in choosing a SaaS solution. Organizations will need to determine whether they are able to utilize a public cloud, or require a private cloud for enhanced security. Additionally, access to support is a significant consideration. While many SaaS options provide 24/7 support, others may only offer support during normal business hours. There will likely be substantive subscription price differences in these offerings, so operating budget may be another factor.

Comparative to premise-based software, SaaS solutions allow organizations to deploy new software rapidly, with a smaller start-up investment. The decrease in implementation time and ability to scale the system means a more timely return on investment (ROI). To gain greater insight into what a SaaS provider is offering, prospective SaaS users should carefully examine the Service Level Agreement (SLA); and ask some very specific questions. Every organization will have different priorities for their SaaS solution and a different tolerance for risk. Whether guaranteed up time, response time and/or security are the most important; a solid SLA will thoroughly address the critical aspects of the contract.

**Keywords:** Lab Management, Laboratory Automation, Laboratory Informatics, LIMS

**Application Code:** Laboratory Management

**Methodology Code:** Laboratory Informatics
As a leading meat processor, Smithfield Foods continually seeks ways to improve quality, process and productivity. The microbiology laboratory sought to eliminate transcription errors, and shorten analysis turnaround and reporting time through automation. The previous system was labor intensive and required re-checking processes for accuracy prior to data release. Automation reduces transcription errors, increases productivity, enhances data quality, and accelerates result delivery. Faster turnaround translates into faster product release, longer shelf-life, and cost savings.

The laboratory leveraged the automated food pathogen detection system to test for Listeria spp., Salmonella spp. and E.coli:0157:H7 on various sample types. Prior to automation, manual steps of loading sample IDs, scanning print outs from instruments, and entering data into reports with secondary review required 40-45 minutes per batch.

The time needed for report review has been reduced to five minutes due to the efficient introduction of technology. Smithfield Foods’ use of automation includes a laboratory information management system (LIMS) to help manage lab data coming from various sources, conduct analysis and generate timely reports that plant personnel use to make critical business decisions. The productivity gains are very compelling – the ability to process Listeria spp. samples has jumped from 700 to 1,400 per day and this is accomplished in 4 hours with 2-3 technicians. The scalability of the LIMS is the primary reason for this increase in processing efficiency and Smithfield looks to achieving additional productivity gains in the future.

Smithfield Foods also believes that the sample tracking and document management capabilities of the LIMS has played a valuable role in helping meet laboratory accreditation requirements.
Environmental testing laboratories face many challenges on a daily basis. This is especially true of those labs that are regulated and need to achieve and maintain certain governmental and industry standards in their testing methods and reporting. Typical examples of regulatory bodies in the environmental sector include the Environmental Protection Agency, state environmental agencies, and federal and state public health organizations. There are also standards bodies in the environmental sector, like the NELAC Institute (TNI), whose mission includes the accreditation of environmental labs. Finally, ISO 17025 is a quality standard that is being adopted by a growing number of environmental laboratories as the basis for accrediting their laboratory quality system.

This presentation will highlight the role of a laboratory information management system (LIMS) in helping the laboratory achieve and maintain compliance with these government bodies and industry standards. We will review a checklist of LIMS capabilities that are specifically designed to ensure regulatory compliance, NELAC accreditation and meeting ISO 17025 requirements. The presentation will also feature a case study of Citizens Energy Group. Citizens Energy is a utility service company providing natural gas, thermal energy, water and wastewater services to 800,000 citizens in the Indianapolis area. Citizens Energy will describe the role that their LIMS has played in helping them meet their regulatory compliance requirements as well as providing other significant benefits which have helped optimize the laboratory’s efficiency.
Development of High Throughput LC/MS/MS Method for Analysis of Perfluorooctanoic Acid from Serum, Suitable for Large-Scale Human Bio-Monitoring

A simple method for determination of perfluorooctanoic acid from human serum by liquid chromatography/tandem mass spectrometry (LC/MS/MS) and utilizing high-throughput sample preparation approach was developed by New York State Public Health Emergency Preparedness Laboratory and used for bio-monitoring studies conducted by New York State Department of Health. 50 µL of serum was mixed with a 0.4 ml of acetonitrile containing isotopically labeled internal standard and phospholipid removal/protein precipitation was performed. Obtained extract was partially dried and analyzed by LC/MS/MS. The linear range of PFOA analysis was 0.5 – 100 ng/ml. LLOQ was selected as 0.5 ng/ml. Method was validated according to APHL guidelines for CLIA laboratories and about 4500 specimens were successfully analyzed using this simple LC/MS method. The low sample volume requirements, limited manual handling of human specimens, high throughput sample preparation and easy upgrade to a fully-automated sample preparation workflow, - all of these make this method advantageous for large-scale epidemiological or bio-monitoring studies.

Keywords: Automation, Clinical/Toxicology, Forensic Chemistry, Liquid Chromatography/Mass Spectroscopy

Application Code: Clinical/Toxicology

Methodology Code: Liquid Chromatography/Mass Spectrometry
Phosphonate salts are petrochemicals commonly used in oilfield for scale inhibition to prevent and control scale problems. Residual analysis is significant to monitor scale treatment and decide retreatment. While multiple phosphonates are used for different wells, traditional techniques, inductively coupled plasma (ICP) and colorimetric analysis, are not able to differentiate phosphonate mixtures in the commingled sample.

In this work, we developed a high sensitive and specific quantitation method for phosphonates in the aqueous matrix by ion chromatography and triple quadrupole mass spectrometry (e.g. IC-MS and IC-MS/MS). Field water sample has high salinity (mostly chlorides) which interferes and suppresses the detection of phosphonate. Phosphonates were separated by hydroxide selective anion exchange column, because phosphonates are multivalent anions which have stronger interaction with the column than the monovalent chloride anions or other anions. Diethylenetriaminepenta (methylene phosphoric acid) (DTPMP), hydroxyethylimino bis (methylene phosphoric acid) (HEMPA) and etheramine phosphonate were used to demonstrate the method. Characterization of molecular ions for each phosphonate was performed and specific selected monitoring reactions (SRM) transitions were preliminarily determined for each phosphonate by using pre-cleaned phosphonate products. Optimization of SRM transitions and method parameters were evaluated by standard phosphonate products and field brine to eliminate internal interferences. Then phosphonate products in sodium chloride synthetic brine were used to validate the method. The technique allows super accurate quantitation of phosphonates with low limit of detection and quantitation for the scale inhibitor residual analysis.

The IC-MS/MS method has a promising application in the energy industry for scale management.

**Keywords:** Ion Chromatography, Mass Spectrometry, Petrochemical

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

**Methodology Code:** Liquid Chromatography/Mass Spectrometry
Liquid chromatography/mass spectrometry (LC/MS) is increasingly used for clinical analysis. LC can separate analytes from potential interferents and reduce matrix effects; however, analysis times can extend to tens of minutes when dealing with complex mixtures such as those involving structural isomers. Ion mobility spectrometry (IMS) has shown the possibility of differentiating isomeric species on a millisecond time scale, which is not possible with MS on its own. Several strategies were developed to improve the IMS separation of metabolites in blood plasma, with the goal of significantly reduced analysis time. These strategies include the use of different cations to form ion complexes with the analytes, but also the use of multimeric complexes and alternative drift gases to enhance structural differences between the isomers.

We have employed these strategies for a wide variety of clinically important analytes, including isomeric steroids, vitamin D, and bile acids (BAs). For example, the isomers chenodeoxycholic acid (CDCA), deoxycholic acid (DCA), hyodeoxycholic acid (HDCA), and ursodeoxycholic acid (UDCA) were extracted by protein precipitation from Red Cross blood plasma and analyzed with an Agilent 1200 series HPLC and Agilent 6560 IM-QTOF instrument.

Ion mobility separation was obtained among the doubly sodiated monomer of the BA isomers, ([M+2Na-H]+, m/z 437.264); calculated collision cross sections were: 199.7 Å² (CDCA), 208.2 Å² (UDCA), 212.1 Å² (HDCA), and 221.4 Å² (DCA). Furthermore, analysis of multimeric species, as well as adducts with alternative cations such as calcium, showed improved separation. For instance, [4M+3Ca-4H]2+ at m/z 842.514 showed baseline separation of CDCA, HDCA, and UDCA, each with a resolving power of at least 60. A 2.2 minute LC-IM-MS run was developed using selected ion complexes to separate these 4 BA isomers in a blood plasma extract. Studies are underway for additional metabolites and different cation adducts.

**Keywords:** Clinical Chemistry, Mass Spectrometry, Metabolomics, Metabonomics

**Application Code:** Clinical/Toxicology

**Methodology Code:** Liquid Chromatography/Mass Spectrometry
The distorted and unique expression of microRNAs (miRNAs) in breast cancer makes them an attractive source of biomarkers for early diagnosis. One of prerequisites for the application of miRNAs in clinical practice is to accurately profile their expression. However, most of the currently available assays for the quantification of miRNAs are ill-suited in complex biological mixtures. Thus, we are the first to develop a LC-MS/MS-based quasi-targeted proteomics assay. This approach actually converted the signal of target miRNAs into reporter peptide by a DNA-peptide probe and the reporter peptide was ultimately quantified using targeted proteomics. In our previous work, the DNA-peptide probe was hybridized with target miRNA, which was immobilized in advance. But this process was susceptible to several potential factors. Thus, we will covalently immobilize the DNA-peptide probe first in this study, followed by peptide sequence adjustment and optimization of immobilization and hybridization conditions. Finally, the developed assay will be applied to determine the amounts of miRNAs in matched pairs of breast tissue samples. The results could facilitate the discovery of new biomarkers for breast cancer early detection.
The extensive use of metal nanoparticles (NPs) has raised great concern due to their adverse effects to the environment and organisms. A method to separate NPs on ultrathin layer chromatographic (UTLC) plate using electrospun nanofibers with charged functional groups as the stationary phase is described in this work. Electrospun nanofibers with ion exchange sites enable the UTLC plates to separate NPs based on charge. The fabrication of electrospun UTLC is simple, fast and inexpensive. The UTLC, with much thinner sorbent layer (10 times thinner than traditional TLC) and very small particle/fiber size (~300 nm), requires minimal mobile phase solvent and provides faster separation and lower detection limit compared to commercial TLC. Moreover, the electrospun UTLC requires no binder between the stationary phase and the solid support, which eliminate band dispersion resulting from interactions with different materials. The electrospun nanofibers show high chemical stability in various mobile phase conditions. The separated NPs are detected by coupling the UTLC with laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS). The analytical performance of this method will be evaluated under optimized condition.

Keywords: Analysis, Chromatography, Nanotechnology, Thin Layer Chromatography
Application Code: Environmental
Methodology Code: Liquid Chromatography/Mass Spectrometry
More than 7 billion tests are performed in clinical laboratories per year. One special class of tests are laboratory developed tests (LDT). LDTs are in vitro diagnostic tests designed, manufactured, and used within a single laboratory, and many are based on LC-MS and LC-MS/MS methods. Vitamin D deficiency has been linked to various conditions such as osteoporosis, hematoma, and cancer. Vitamin D deficiency is clinically confirmed by measuring the major circulating form, 25-hydroxy vitamin D2 and D3 (25OHD2 and 25OHD3, respectively) in the patient serum.

A customer, using aliquot tubes from a specific source, received unusual results from a LC-MS/MS LDT for 25OHD2/3 determination. The customer commented that these tubes were known to cause ion suppression. Our group investigated the customer findings to determine if an extractable from the aliquot tubes causes this issue.

Using acetonitrile, oleamide was extracted from the tube walls. Comparison to other tubes showed these specific tubes contained much higher amounts of oleamide. Co-injection of the sample tube extract or oleamide with 25OHD2/3 samples followed by LC-MS (using the same LC method) resulted in co-elution and concomitant ion suppression of the 25OHD2/3 peak. Because the LDT used LC-MS/MS, they were unable to observe this co-elution. These results caused the tube supplier to change their formulation. Subsequent studies showed that oleamide was not extractable from the new formulation, and therefore use of these tubes no longer caused 25OHD2/3 ionization suppression.

Keywords: Bioanalytical, Clinical Chemistry, Liquid Chromatography/Mass Spectroscopy, Polymers & Plastics
Application Code: Clinical/Toxicology
Methodology Code: Liquid Chromatography/Mass Spectrometry
Electron Ionization (EI) LC-MS is highly desirable as it enables automated library based identification and is free of any ion suppression effects that plague ESI and/or APCI. Thus, bringing back EI to LC-MS is highly valuable if a robust, simple to operate and low cost EI-LC-MS system can be designed and built.

We developed a new electron ionization (EI) LC-MS with Cold EI system based on an Agilent 5977 quadruple GC-MS. Cold EI is EI of vibrationally cold molecules in supersonic molecular beams (SMB). The LC effluents are pneumatically sprayed followed by fast thermal vaporization. The vaporization chamber is connected to the supersonic nozzle via a capillary flow restrictor to suppress cluster formation.

A fly-through ion source was used and the ions were mass analyzed by the 5977 mass analyzer. Cold EI results in enhanced molecular ions which are often weak or missing in standard EI, yet it is fully compatible with library identification and it uniquely provides:

1. Library identification with compounds names and structures
2. Extensive fragment MS information
3. Our TAMI molecule identifier software enables the elucidation of elemental formula
4. The fly-through ion source eliminates any ion source tailing and degradation
5. Non-polar compounds analysis is enabled
6. Cold EI provides uniform, compound independent ionization efficiency
7. Cold EI does not suffer from any ion suppression or enhancement effects

Our system was explored in the analysis of cannabinoids in Cannabis flowers, in the analysis of anti-oxidant compounds and cholesterol, free fatty acids and lipids in serum.

Keywords: Instrumentation, Liquid Chromatography/Mass Spectroscopy
Application Code: General Interest
Methodology Code: Liquid Chromatography/Mass Spectrometry
Pharmaceutical Analysis and Stability

# Novel Method for Determining Shelf-Life Stability of Peptides

Peptides are small amino acid chains that can be used as active pharmaceutical ingredients in drug products. The Accelerated Stability Assessment Program (ASAP), which has been widely applied to small molecule drug products, was applied for the first time to a pharmaceutically active peptide, the antibiotic bacitracin. Bacitracin and its complex with zinc were exposed to temperature and relative humidity conditions from 50 to 80°C and from 0 to 63% for up to 21 days. High-performance liquid chromatography was used to analyze the stressed samples for both degradant formation, loss of the active (bacitracin A) and two inactive isoforms, with identities confirmed by mass spectrometry. These data were then analyzed using a humidity-corrected Arrhenius equation and isoconversion (time to the failure point) approach to create a shelf-life predicting model for typical storage conditions. Model fitting was found to be good with low residuals in both temperature and relative humidity axes for all parameters examined. The generated models’ predictions for both the native and zinc complex of bacitracin for both formation of the major degradation product (F, the oxidative deamination product) and loss of the active isoform (A) were consistent with longer-term measured values at 30°C/53%RH and 40°C/75%RH, validating this approach for accelerating the determination of long-term stability of a peptide. The modeling also provides insight into the origin of stabilization of bacitracin by zinc, which appears to limit mobility (reduce collision frequency) at the reactive site.

**Abstract Text**

Peptides are small amino acid chains that can be used as active pharmaceutical ingredients in drug products. The Accelerated Stability Assessment Program (ASAP), which has been widely applied to small molecule drug products, was applied for the first time to a pharmaceutically active peptide, the antibiotic bacitracin. Bacitracin and its complex with zinc were exposed to temperature and relative humidity conditions from 50 to 80°C and from 0 to 63% for up to 21 days. High-performance liquid chromatography was used to analyze the stressed samples for both degradant formation, loss of the active (bacitracin A) and two inactive isoforms, with identities confirmed by mass spectrometry. These data were then analyzed using a humidity-corrected Arrhenius equation and isoconversion (time to the failure point) approach to create a shelf-life predicting model for typical storage conditions. Model fitting was found to be good with low residuals in both temperature and relative humidity axes for all parameters examined. The generated models’ predictions for both the native and zinc complex of bacitracin for both formation of the major degradation product (F, the oxidative deamination product) and loss of the active isoform (A) were consistent with longer-term measured values at 30°C/53%RH and 40°C/75%RH, validating this approach for accelerating the determination of long-term stability of a peptide. The modeling also provides insight into the origin of stabilization of bacitracin by zinc, which appears to limit mobility (reduce collision frequency) at the reactive site.

**Keywords:** Amino Acids, Bioanalytical, Peptides, Pharmaceutical

**Application Code:** Pharmaceutical

**Methodology Code:** Liquid Chromatography/Mass Spectrometry
Neonatal hypotension affects 50% of patients within NICUs, and is commonly treated with inotropes such as dopamine and dobutamine. Our unit reports instances of reduced mean arterial pressures (MAP) towards the end of 24h infusions, occasional IV line discolouration, and rapid spikes of MAP upon the start of new infusions. In this study we investigated the possible effects of temperature and ambient lighting on drug stability during continuous infusion. Syringe-driver infusions were set up of either dopamine or dobutamine, diluted with either 0.9% normal saline (NS) or 5% glucose, delivering 3mcg/kg/min and 5mcg/kg/min respectively via 206cm extension sets. Infusions were run over 24 hours protected or exposed to ambient lighting and approximately half the tubing was incubated at 35°C. Concentration was measured using cyclic voltammetry, at the start and end of infusion, collecting samples from the syringe and infusion terminal. Our findings suggest that greater error is introduced following preparation of infusions utilising 1mL syringes. A significant decrease in the concentration of dopamine after 24 hours was observed when prepared in 0.9% NS. Dobutamine shows no significant degradation when prepared in 0.9% NS or 5% glucose over 24 hours. This study finds significant inotrope degradation over a 24 hour intravenous infusion. Much more research is needed to elicit the multifactorial mechanisms behind the harmful MAP fluctuations in treating neonatal hypotension of which degradation could be a potentially instrumental factor.
ML-163 Topical Solution for Cats contains three Active Pharmaceutical Ingredients (APIs): praziquantel, (S)- enantiomer of afoxalaner, and eprinomectin. A reversed phase high performance liquid chromatography (RP-HPLC) method has been developed for release and stability testing of the ML-163 Topical Solution for cats. The topical solution is dissolved in a diluent composed of 70% Acetonitrile (ACN) and 30% water (H2O) (v/v). Analytes were eluted on ACE 5 C18 (100 mm x 4.6 mm i.d., 5 µm particle size) maintained at 35 °C in an HPLC system with gradient elution using mobile phases containing 0.05% phosphoric acid (H3PO4) in water as mobile phase A and Methanol as mobile phase B and Acetonitrile (ACN) as mobile phase C. All analytes of interests were fully separated within 46 minutes. Analytes were detected with UV detection at 245 nm and quantitated against a single external reference standard with a quantitation limit of 0.3% of their respective Label claim. The method was demonstrated to be accurate, robust, specific, and stability indicating.
Synthetic peptides are one of the fastest growing market sectors in the biopharmaceutical industry. Because of their high specificity and stability, synthetic peptides are candidates for the treatment of a wide variety of diseases. Most synthetic peptides are synthesized by solid-phase peptide synthesis (SPPS), a process in which individual amino acids are linked through consecutive coupling steps on a solid support. The resulting product is a complex mixture containing not only the desired peptide, but also peptides having amino acid insertions and deletions as well as a variety of additional byproducts. Like with any pharmaceutical product, rigorous characterization and purification is needed to ensure safety and efficacy. Quality control analyses of peptides are traditionally carried out using reversed-phase LC methods with optical detection. While this provides a basic level of insurance, by incorporating mass detection as an orthogonal technique, improved sensitivity, expanded detection limits, and increased confidence about peak homogeneity are achieved. In this study, we present a strategy for the acquisition of optical data with added mass detection for enabling identification of a target peptide, eledoisin, and its impurities. This work demonstrates the ability to improve productivity and confidence in a production environment by combining optical detection and a simple and cost-effective strategy for mass detection in a single workflow.

Keywords: Chromatography, Peptides, Pharmaceutical, Quadrupole MS
Application Code: Pharmaceutical
Methodology Code: Liquid Chromatography/Mass Spectrometry
MS has been used as an effective tool to detect and identify small molecule drug metabolites, as any biotransformation leading to elemental composition change can be readily observed with an m/z change. For large molecules such as peptides, mAb, and proteins, similar modifications are much harder to detect, due to: (a) modifications occurring to a large biomolecule would be relatively small; (b) the ions species with and without the modification are typically hard to separate with chromatography; (c) the MS resolving power at high m/z are poor; or (d) the widely used ESI will create multiply charged ions, further diminishing the MS differences. We will demonstrate that spectral accuracy, not the conventional mass accuracy, is the key in accurate detection and relative quantitation of biomolecule modifications from peptide deamidation to protein glycan adducts.

For deamidation, the overlap of the 13C isotope from the native with the monoisotope peak of the deamidated peptide can compromise MS detection and quantitation, requiring either full chromatographic separation or very high MS resolving power. Complete chromatographic separation, while possible, is time consuming and tedious, and the separation of a pair of triply charged deamidated (m/z = 849.0433, C114H159N27O40) and native (m/z = 848.7153, C114H160N28O39) peptide requires a MS resolving power of 400K, above the ability of most commercially available MS. We propose a different approach to detect and differentiate these modifications through a spectral accuracy calibration process involving MS peak shape. This calibration is then applied to the overlapped spectrum of deamidated and unmodified peptide mixture. Since the measured MS is a linear combination of the deamidated and unmodified peptides, a linear regression can be used to obtain the relative concentration of the deamidated peptide, even when it is not chromatographically separated.

Keywords: Biopharmaceutical, Liquid Chromatography/Mass Spectroscopy, Mass Spectrometry, Time of Flight
Application Code: Pharmaceutical
Methodology Code: Liquid Chromatography/Mass Spectrometry
Volatile organic reagents are commonly used in the production of active pharmaceutical ingredients (APIs) and are not always fully removed by the manufacturing process. These residual solvents may have toxicity concerns and need to be controlled below levels prescribed by ICH guidelines. Pharmaceutical labs typically use API specific GC methods where only solvents used to manufacture that API are controlled. If the manufacturing process changes, as it often does during clinical development, the method may need to be re-developed and re-validated. A more efficient approach is to use a universal method covering the common solvents used in API manufacturing and apply this across all API projects. This eliminates the need for individual method development and can greatly streamline validation activities.

Developing a universal method is not trivial, as it needs to cover a large number of solvents and should show applicability over multiple development projects. Here we describe a universal GC method specific for 41 solvents that are routinely used in API production. We have used this approach successfully for multiple APIs produced in our plant over several years. This method uses dual FID/NPD detection to cover the heavier nitrogen-containing solvents like TEA, DIPEA, DMF, DMA, and NMP which are difficult to analyze using FID alone. We will describe the method in detail and provide simple guidelines for its use in controlling residual solvents. Examples that illustrate the process from method selection to validation will also be discussed to allow other scientists to apply this approach in their lab.

Keywords: Gas Chromatography, Method Development, Pharmaceutical, Volatile Organic Compounds
Application Code: Pharmaceutical
Methodology Code: Gas Chromatography
The development of the pharmaceuticals brought along great change in human health. However, these pharmaceuticals are only helpful if they are free from impurities and are administered safely. Likewise, how drugs are packaged are also important. Packaging must also keep drugs free from contamination, slow microbe growth, and ensure the product is safe through its shelf life. Also, the packaging material itself must not contaminate the pharmaceutical.

To ensure that these drugs serve their purpose, various instrumental methods are used to analyze them. These analytical techniques include titrimetric, spectroscopic, and chromatographic. Of the chromatographic techniques, GC/MS, used for detection of volatile and semi-volatile organic compounds, has been used to determine drug amount as well as detecting residual solvents and process related impurities.

High-molecular weight products such as polypeptides, or thermally unstable antibiotics, or other polymers associated with the drug, or in drug packaging, limit the scope of this technique. Adding Pyrolysis to GC/MS can be used to study not only thermally labile solvents, additives and impurities, but polymeric material as well. Using high temperatures to break molecular bonds, polymers are broken apart into volatile fragments in a repeatable way so that it can be sent to a gas chromatograph for analysis. Using sub-pyrolysis and pyrolysis temperatures to introduce solid samples to a GC/MS, various brands of over the counter medications and their packaging will be compared, looking at which include residual solvent, any organic fillers, and active ingredients.

Keywords: GC-MS, Pyrolysis
Application Code: Pharmaceutical
Methodology Code: Gas Chromatography/Mass Spectrometry
Protein therapeutics, including antibodies, is perhaps the most rapidly growing sector of the pharmaceutical industry. Key in the production of antibodies is the ability to assess the growth process in real time. Widely known for its high affinity for the Fc region of IgG, of recombinant Staphylococcus aureus protein A (rSPA) has long been utilized to capture IgG from complex biological matrices such as cell culture supernatant. Combining the attributes of both separation and immunoassay techniques, affinity chromatography employing protein A is typically the first chromatographic step in the downstream processing of therapeutic monoclonal antibodies (mAb). Moreover, at the analytical scale, this technique is also used in the early development phase of mAb production where high-titer harvest cell culture samples need to be screened and monitored for IgG expression.

This study investigated the potential application of the rSPA-modified capillary-channeled polymer (C-CP) fiber columns to the rapid, highly selective, quantification of IgG in complex biological matrices. Optimization of the chromatographic method regarding mobile phase components and loading/eluting conditions was investigated. The six-minute analysis, including a loading step with 12 mM phosphate pH 7.4, an elution step with 0.025% phosphoric acid and a re-equilibration step, was optimal for quantification of IgG1 from 0.075 to 3.00 mg mL-1. The inter-day and intra-day precision of the method were 3.1% and 3.5% respectively. Column performance was decently reproducible across columns prepared from different batches. The method appears to be well suited for high IgG titer analysis in complex matrices.
The emergence of protein-based drugs brings along new challenges in term of stability and formulation. Proteins have a strong tendency to denature depending on parameters such as temperature, shear, solvent, etc. This instability affects the shelf life and can alter the drug efficiency but can be observed because of viscosity variation of denatured protein. We propose a new rheological approach with visual instrument FLUIDICAM to simply determine the viscosity as a function of shear rate and temperature in a single experiment set-up. Using a small sample volume, the technology allows flow viscosity measurements of liquid products from water-like inks to thick cosmetic formulations with sample volumes of hundreds of microliters.

During the measurement a sample and a viscosity standard are pushed together through a microfluidic chip (Y-junction) at controlled flow rates. Images of the resulting laminar co-flow are acquired with digital camera and the position of the interface is measured. This position is related to the viscosity and the ratio of flow rates between the sample and the reference allowing to determine the viscosity.

In this work, the impact of protein concentration on viscosity was measured. Bovine serum albumin was used at concentrations from 25 to 250 mg/ml at shear rates up to 100 000s-1. No shear related denaturation (unfolding) could be observed at these conditions. Then, the impact of stabilizing additives was studied to determine the ability to protect the protein from denaturation. Fresh BSA solutions were prepared with various additives (Arginine, Histidine, NaCl) and then submitted to a thermal stress (40°C). Using Fluidicam viscosity was measured with enough precision to discriminate the smallest variation in the sample thus allowing to rapidly evaluate the impact of additive on protein stability.

Keywords: Lab-on-a-Chip/Microfluidics, Protein, Rheology
Application Code: Pharmaceutical
Methodology Code: Physical Measurements
When formulating and deconstructing tablets, accurately determining API particle metrics, coating thicknesses, distribution, and polymorphic form is critical. With the majority of APIs having a particle size below 30 micrometres, producing this information requires high spatial resolution (down to 1 micrometre) and high chemical specificity so different polymorphic forms are revealed. Maintaining the integrity of particle and granule domains is hugely desirable to ensure subsequent statistics from analyses are representative of the contents. Naturally cleaving the tablet surface, which is not mechanically altered via microtoning or milling, achieves this and drastically simplifies the sample preparation process.

Until now raw cleaved surfaces could not easily be analysed by Raman spectroscopy at high spatial resolution as the high magnification data collection lens, required to produce high spatial resolution, inherently exhibits a small depth of field and is extremely sensitive to focus changes. Live focus tracking, working in parallel with fast Raman imaging techniques such as StreamLine, now enables such information even where significant topographic variation is present.

In this work, we have applied a new real-time micro-Raman focus tracking technique, LiveTrack, implemented on a new pharmaceutical dedicated Raman system, to a series of different cleaved tablets. The resulting high spatial resolution data enables detailed information on the API composition, coatings, domains and distribution – providing a comprehensive and realistic deconstruction of the tablet. Here we discuss and present data on a range of pharmaceutical examples including:

- Small API domain size cleaved tablet deconstruction
- Pre-formulation granule blend analysis
- Entire cleaved tablet deconstruction
- Snapped tablet deconstruction

Keywords: Pharmaceutical, Raman Spectroscopy, Spectroscopy
Application Code: Pharmaceutical
Methodology Code: Vibrational Spectroscopy
Applying Quality by Design (QbD) principles to impurity investigations and control strategies requires a thorough understanding of the manufacturing process. The drug development lifecycle is lengthy and complicated, with multiple stakeholders, often in disparate locations around the globe. During the process, a vast array of complex and heterogeneous data is collected and transferred between the various project teams. Many of these groups have their own systems for capturing data, but a frequently reported problem is that no single centralized system exists which is capable of linking the various systems together. As a result, data sharing between groups and individuals is often relegated to an Excel or PowerPoint document on SharePoint. Individual users are expected to generate their own documentation for sharing, resulting in inconsistencies. Efficiency is greatly reduced as those not intimately involved with the results cannot have confidence that they adequately understood what has been communicated.

Here we present an approach utilizing a combination of software, algorithmic tools, and databases to assist in the unification of process-related, structural and analytical data to allow cross-functional teams to effectively communicate and process related knowledge. The result is a living/working tool that can grow over the development lifecycle—from Discovery, through Development, and to Launch—and allowing the visualization of many discrete process-related variables in a single, searchable interface for improved decision-making and faster qualification of the drug product.

Abstract Text

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Keywords: Data Analysis, Informatics, Method Development, Scientific Data Management
Application Code: Pharmaceutical
Methodology Code: Laboratory Informatics
Evaluation of the Nicotine Particle Size in an Aerosol Formed by an Electronic Cigarette

Electronic cigarettes (e-cigarette) are popular drug delivery systems. An aerosol is produced by the e-cigarette when the e-liquid passes over the heated coil, vaporizes, and then condenses with water in the atmosphere. The size of the droplets formed in the aerosol can vary. The size of the particle is a major factor in determining where and if that particle will deposit in the lung. The objective of this research was to evaluate whether or not a typical electronic cigarette, KangerTech AeroTank, 1.8 preassembled atomizer, and an eGo-V2 at variable voltage battery, is capable of producing an aerosol with a significantly small enough particle of an active drug, nicotine, to be deposited in the lung for absorption in the blood stream.

A 12 mg/mL nicotine 50:50 PG:VG e-liquid formulation was aerosolized 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 to 18 µm. The concentration of nicotine on each stage of the MOUDI was determined using an HPLC-MS/MS system. This experiment was performed at three different voltages of 3.9, 4.3, and 4.7 V on the electronic cigarette.

The percent of recovered nicotine was determined for each stage. Stages 7 and 8, representing particle size ranges of 0.54 to 0.31 and 0.31 to 0.172 µm respectively, consistently collected approximately 33% of the aerosol. Additionally, all 3 voltages produced ultrafine particle sizes, <0.1 µm. Compared to traditional cigarette smoke, the aerosol produced by e-cigs has similar particle size production, centered at 0.3 µm (±), but also produced more ultrafine particles than a traditional cigarette. Therefore, this type of electronic cigarette is capable of producing small enough particles with nicotine, to be deposited in the lung for absorption into the blood stream.

This research was supported the National Institute of Justice, Award 2014-R2-CX-K010

Keywords: Aerosols/Particulates, Forensics, Liquid Chromatography/Mass Spectroscopy, Particle Size and Distribution
Application Code: Pharmaceutical
Methodology Code: Liquid Chromatography/Mass Spectrometry
A fully automated 2D barcode sample tracking system is evaluated for regulatory-based sample preparation and ICP/ICPMS analysis. The system uses 2D barcoded sample and digestion tubes to store, reference and transmit data to LIMS (Laboratory Information Management Systems) for report generation. The commercially available PlasmaTraxTM consists of four distinct stations integrated with one complete software package. 1) TraxGPTM is a mobile handheld scanner used at the point of sampling to record collection time, GPS location, sample type, etc. 2) TraxMassTM is a scientific balance with an integrated barcode scanner for recording sample weights. 3) TraxPrepTM is an offline sample preparation station which references barcoded information to prepare samples according to regulator or user-defined protocols. 4) TraxSCTM is an autodilution platform which utilizes barcoded sample type and preparation information to build appropriate calibration curves and intelligently dilute and analyze samples by ICP/ICPMS.

The ability of PlasmaTraxTM to automatically perform a complete USP <232> and <233> protocol is investigated for a variety of oral drugs. The daily dose and sample mass of oral drugs ranges from 0.01-20.0 g/day and 0.180-0.220 g, respectively. The system automatically calculates and prepares unspiked and J-spiked solutions according to USP oral PDE (Permissible Daily Exposure) limits. The autodilution system selects the correct J standard (High-J or Low-J) and calibrates at the 0.5 and 1.5J concentrations defined by each drug's daily dose. The final results indicate samples are run according to USP protocol and pass validation criteria of accuracy, repeatability, suitability, and specificity.

Keywords: Drugs, Mass Spectrometry, Scientific Data Management, Trace Analysis
Application Code: Pharmaceutical
Methodology Code: Mass Spectrometry
Citrate and phosphate are important components in many pharmaceutical products. For example, citrate has been used in anticoagulants to preserve blood and to prevent excess bleeding during rectal enema treatments, citrate is added as a flavoring and stabilizing agent to mask the taste of medicinal flavors, and both citrate and phosphate are counter ions which affect the Active Pharmaceutical Ingredient (API) solubility and subsequently impacts efficacy and toxicity. The benefits of using ion chromatography (IC) methods to determine small ions are well known. An IC method has been adopted by The United States Pharmacopeia (USP) in General Chapter <345> for the determination of citrate and phosphate in pharmaceutical products. However, there has been much technological advancement in IC since the development of the method in <345>. Here we demonstrate an improved citrate and phosphate assay that reduced run times from 10 to 5 minutes and sample injected from 10 μL to 2.5 μL. Using a higher porosity and smaller particle size L81 column, which has similar selectivity to the USP <345> specified L61 but with higher peak efficiency and capacity, this faster assay delivered higher analyte sensitivities (LOQs 0.03 and 0.06 mg/L of phosphate and citrate) and extended linear range. This assay can be executed on an IC system that features eluent generation, thus removing the possible errors associated with mobile phase preparation and increasing reproducibility within and between labs.
Pharmaceutical Characterization

Core-Shell vs. Fully-Porous Particles for High Throughput Analysis

As the demand of higher productivity and the complexity of HPLC analyses increase, the quest for higher resolving power and reduced analysis time led to the development of HPLC sorbent with incrementally smaller particle size in the past decades. The use of HPLC columns packed with sub-2 µm fully porous silica sorbent has been shown to provide the desired benefits, but it requires the use of dedicated UHPLC systems because of the back pressure it generates. In the past 5-7 years, a new development approach to the silica particle morphology has led to the commercialization of core-shell materials which offer higher efficiencies than fully porous silica particles of the same size. Many publications have been dedicated to the comparison between the fully porous particles and the core-shell particles in their performances in different applications, as well as potential issues such as mechanical strength, sample loading capacity, particle scalability, ease of method transfer and required system optimization. It is the purpose of this presentation to summarize such comparisons, as well as to provide a glimpse into the future potential of these two approaches to particle morphology in various modes of separation.

Keywords: HPLC, HPLC Columns, Liquid Chromatography/Mass Spectroscopy
Application Code: Pharmaceutical
Methodology Code: Liquid Chromatography
Pharmaceutical Characterization

Fast Centrifugal Partitioning Chromatography

Centrifugal Partition Chromatography (CPC) instrumentation is used in the natural products, purification in academia and industry as a means of fractionating complex mixtures of organic components and achieving high recoveries and purities of biomarkers to use as reference standards, APIs etc. Kromaton’s Fast Centrifugal Partition Chromatography (FCPC) offers several advantages versus traditional methods such as HPLC in that no solid packing material is required, which is complicated to dispose of and can cause irreversible adsorptions and cross contaminations. The FCPC is a discrete stage-wise device that uses the specific partitioning coefficients of the individual components for isolation of the product fractions. Due to the large quantity of extraction stages in the rotating column (theoretical plates), components with similar molecular structures can be easily isolated. Recent improvements in the stage cell design, such as twin-cells versus traditional Z-cell profiles improve diffusion and ensure high stationary phase retention within the cells. Twin cell designs have been developed that improve resolution and degree of fractionation. FCPC technology is commonly used in isolation of botanicals’ metabolites and applications include Cannabinoids purifications, Tobacco natural compounds, Opiate Derivatives, and other substances that can be used in Drugs, Phytotherapy, Nutraceuticals and cosmetics. Capacities range from the milligram scale for analytical scale quantities, up to preparative scale quantities in the gram scale, and production scale capacities are available for kilogram scale fraction collection. Developments have been made in the detection and sample collection system software. Manual systems are available or they can be used in conjunction with a PLC. Commonly used solvent systems include the Arizona range, HeMWat, aqueous biphasics, generally from very polar to non-polar solvent pairs.

Keywords: Biopharmaceutical, Chromatography, Liquid Chromatography/Mass Spectroscopy, Natural Products
Application Code: Pharmaceutical
Methodology Code: Liquid Chromatography
Military members are exposed to unique operational environments that include toxic industrial chemicals and materials. Capturing these exposures using current methods is often problematic due to supply logistics, power requirements, equipment ruggedness, and shipping to analytical labs outside of theater. Research to provide a low cost, rugged, sensitive, specific, small, lightweight dosimeter that can integrate doses over time will be presented. This dosimeter will provide information for exposure records.

A specially prepared silicon wristband (SWB) was evaluated for 45 volatile organic compounds (VOCs) including those found in aircraft exhaust (i.e., JP-8 and BTEX) and in aircraft lubricants/coolants (tri-organophosphates/poly alpha olefins). SWBs were infused with VOCs/semi-volatile organic compounds (SVOCs). SVOCs were then recovered via extraction and VOCs were determined by thermal desorption. All analytes were analyzed by gas chromatography mass spectrometry. Studies of the effects of shipping were performed and SWBs were infused and stored at 4°C for 7 d. Stability studies were also performed.

All compounds were successfully infused into the SWB. The average VOC recovery was 95% with a relative standard deviation of 4%. SWB transport and stability after 7 d at 4°C averaged 107% for VOCs and the relative standard deviation was 7.9%. Stability of the infused SWB after 27 d averaged 108% with a relative standard deviation of 6.0%.

The SWB shows excellent promise as an approach to recover multiple classes of Air Force relevant VOCs/SVOCs. The transport and stability of VOCs in the SWB will add to their use and utility under challenging conditions. Future testing will expand the collection effort to other compounds and operational field testing will be conducted.

Keywords: Industrial Hygiene, Method Development, Semi-Volatiles, Trace Analysis

Application Code: Industrial Hygiene

Methodology Code: Sampling and Sample Preparation
Sampling and Sample Preparation - Liquid Extraction, SPE and Others

Development of a New Pressurized Liquid Extraction Method for Extracting Analytes from Fatty Matrices

Pressurized liquid extraction has been shown to be useful in a variety of different applications including food and food safety. In these markets the sample preparation of fatty matrices can be particularly difficult. Sample preparation is the key to minimize any matrix effects in the analytical measurement. We present a new pressurized liquid extraction method and the effectiveness of different sorbents for matrix cleanup. The effects of five different sorbents were analyzed and compared to different liquid extraction techniques. The sorbents were found to be more effective with the proposed new liquid extraction method than traditional methods.

Abstract Text

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Keywords: Accelerated Solvent Extraction, Extraction, Lipids, Sample Preparation
Application Code: Food Science
Methodology Code: Sampling and Sample Preparation
Perfluorinated compounds (PFCs) are of increasing concern as they are detected in environmental and human samples. Originally thought to be inert compounds, they are long-lived and may cause tumors and endocrine effects. They bioaccumulate so continued exposure may be especially hazardous. The measurement of PFCs was included in the Unregulated Contaminant Monitoring Program 3 (UCMR-3) and occurrence evaluated in drinking water across the US. US EPA Method 537, which passes 250 mL of water through a cartridge and subsequent analysis with LC/MS/MS, was developed to support this effort. As reports of PFC contamination continue to draw headlines, the need for a simple and automated analysis becomes more critical.

This work evaluates the development of automated methodology for EPA 537. Background levels and the need to develop a system that will minimize contamination will be discussed. A range of water samples will be analyzed and challenges and results presented.

Keywords: Environmental Analysis, Liquid Chromatography/Mass Spectroscopy, Solid Phase Extraction
Application Code: Environmental
Methodology Code: Sampling and Sample Preparation
## Abstract Text

Red blood cell (RBC) transfusion is a therapeutic intervention performed frequently (> 40,000 units daily) in hospitals and other clinical institutes. Although major shortages of RBC supplies in general use have become uncommon, adverse post-transfusion complications still hinder this life-saving activity. The RBC storage lesion, the physical and metabolic changes that occur to RBCs during storage, may be a major contributor to post-transfusion complications. Previously, our group has proposed a normoglycemic version of current standard storage solutions and shown the benefits of low glucose storage through various quantitative determinations of such RBC properties as improved cell deformability and ATP release, and decreased sorbitol production. It is essential to maintain the glucose level of the stored RBCs in a normalglycemic range (4-6 mM) without complicating the overall storage process. Here, we hypothesize that a 3D-printed, intravenous (IV) piggyback system will maintain normoglycemic RBC storage. The device is designed to have a container where concentrated glucose saline solution is preserved, and a single switch allowing controllable release of glucose to “leak” into the stored RBCs. Preliminary results have shown the glucose concentration of normoglycemic RBC storage ranging from 4.00 ± 0.04 mM to 5.25 ± 1.72 mM (mean ± stdev, n = 4) in a 35-day storage, which highly suggests that the printed device enables the control of the glucose level in the stored RBCs without exposing the stored cells to external conditions.

## Keywords:
- Biological Samples, Biomedical, Sample Handling/Automation, Sample Preparation

## Application Code:
- Clinical/Toxicology

## Methodology Code:
- Sampling and Sample Preparation
Nucleic acids are biopolymers that constitute important diagnostic molecules for a broad range of applications from clinical testing to forensic analysis. A major challenge faced by DNA and RNA analysis techniques is the instability of nucleic acids toward enzymatic degradation by nucleases that are often present in biological samples or the surrounding environment. In this study, we investigated hydrophobic magnetic ionic liquids (MILs) as nucleic acid preservation media and evaluated their compatibility with downstream amplification methods. DNA samples stored in the benzyltrioctylammonium bromotrichloroferrate(III) ([N888Bn+][FeCl3Br]) and trihexyl(tetradecyl)phosphonium tetrachloroferrate(III) ([P66614+][FeCl4]). MILs and incubated with deoxyribonuclease I (DNase I) retained their molecular weight for up to 72 h at room temperature, whereas DNA treated with DNase I in aqueous solution underwent complete degradation. The [N888Bn+][FeCl3Br] and [P66614+][FeCl4]. MILs were also found to enhance the resistance of plasmid DNA (pDNA) toward nuclease degradation. pDNA samples treated with 20 units of DNase I and incubated at room temperature for 72 h were successfully amplified using polymerase chain reaction (PCR). The preservative capabilities of MILs were also investigated for RNA samples treated with the ubiquitous enzyme, ribonuclease A (RNase A). While RNA in aqueous solution was completely degraded by RNase A, storage of RNA within MIL solvent enabled its detection by reverse transcription PCR (RT-PCR). Using a hydrophobic MIL solvent, a magnet-based workflow was established in which mRNA was extracted from yeast cell lysate, protected from enzymatic degradation during storage, and subsequently amplified using RT-PCR.

Keywords: Extraction, Nucleic Acids, Sampling
Application Code: Bioanalytical
Methodology Code: Sampling and Sample Preparation
Solid phase microextraction (SPME) techniques have long been presented as environmentally friendly, fast, automatable and therefore green alternatives to classical solvent-based sample preparation methods. As a solventless approach, SPME possesses an inherent advantage when used for environmental analysis as it does not contribute to the environmental problem it is intended to detect. Despite these advantages, skeptics of SPME have alleged that the technique may lack the accuracy and sensitivity of traditional exhaustive extraction methods.

With these skepticisms in mind, this proposed work presents a highly sensitive and accurate thin film microextraction (TFME) method as a green alternative to LLE for the determination of 23 multi-class pesticides from surface water samples. The implementation of polydimethylsiloxane/divinylbenzene (PDMS/DVB) and PDMS/DVB-carbon mesh supported membranes, in lieu of fiber-based SPME, offers order(s) of magnitude enhancements in sensitivity. This signal improvement was demonstrated by method limits of detections (MLOD’s) in the low ng L⁻¹ range for most of the pesticides studied while only requiring 30 mL of sample when analyzed on a thermal desorption unit (TDU) equipped single quadrupole GC-MS instrument. Furthermore, these MLOD’s were shown to be at least 10 times lower than those achieved using an EPA certified, LLE method performed at an accredited analytical laboratory participating in the study. Moreover, the method accuracy was also validated through double blind split analyses of 18 surface water samples. Good agreement between the two methods was achieved with accuracy values between 70-130%, for the majority of analytes. The comparison of TFME and LLE demonstrated that the novel TFME approach gave similar accuracy to LLE, while providing additional advantages including higher sensitivity, small sample volumes, and faster throughput. Given these advantages, TFME shows great potential for adoption as a green alternative to LLE.

Keywords:
- Environmental Analysis
- Gas Chromatography/Mass Spectrometry
- Pesticides
- SPME

Application Code: Environmental

Methodology Code: Sampling and Sample Preparation
Surface plasmon resonance (SPR) spectroscopy is a label-free method for observing biomolecular binding events in real time with sensing features. Our research objective is to achieve ultra-low level detection of small molecules that are critical markers of cancer diseases. Also, we are interested in monitoring pH changes in less abundant biological samples. As we know, pH is an essential parameter in biological, medical, and industrial applications. Monitoring the pH level in biological fluids can indicate abnormal conditions. We will present our findings on sensitive detection of small molecules and amplification of SPR signals by using chemical indicators and detection by an SPR microarray imager. In addition, we applied our method to measure low levels of formaldehyde in urine.

Acknowledgements. This project was supported by Oklahoma State University.

3. Kick, A.; Mertig, M., Characterization of pH-sensitive polymer layers by surface plasmon resonance and quartz crystal microbalance. Physica Status Solidi (a),1-5, 2015,
There's an unmet need to develop a robust, inexpensive, and simple sensor to detect blood phenylalanine (Phe) levels to improve the treatment of phenylketonuria patients who need regular blood Phe level monitoring. We developed a 2D photonic crystal (2DPC) sensing material for detection of phenylpyruvate (PhPY), an enzymatic by-product of Phe. This sensor utilizes 2D arrays of polystyrene particles embedded in a volume responsive hydrogel. The hydrogel is synthesized via copolymerization of 2-hydroxyethyl acrylate, acrylic acid, and tert-butyl (2-acrylamidoethoxy)carbamate (TBAC). The oxyamine group of TBAC reacts with PhPY to form a covalent bond via oxime ligation which binds additional negative charges on the polymer system, generating a volume phase transition (VPT) in the hydrogel. PhPY induced swelling alters the spacing of the embedded 2DPC. The degree of swelling is proportional to the PhPY concentration. The 2DPC spacing change can be easily calculated from Debye diffraction ring diameter measurements using a simple green laser pointer. The magnitude of particle spacing change is proportional to the PhPY concentration. This un-optimized 2DPC sensor showed a particle spacing change of ~60 nm for 5 mM concentration PhPY at pH ~5.3 in acetic acid buffer solution within 30 minutes. This proof-of-concept sensing motif of PhPY is being developed for monitoring enzymatically produced PhPY in blood obtained by fingerstick.

Keywords: Bioanalytical, Biosensors, Detection, Sensors
Application Code: Bioanalytical
Methodology Code: Sensors
MicroRNAs (miRs) are important regulators of gene expression and are involved with the progression of various diseases. When attempting to detect a single miR intracellularly, there can be several other naturally occurring miRs that have similar sequences to the miR of interest. The variation in sequence between analyte miR and off-analyte miRs can be as little as a single nucleotide difference. Often these small differences in sequence cause false-positive signal because of their overall similarity to the analyte sequence. Our double-stranded reporter+probe biosensor uses toehold-mediated strand-displacement to initiate analyte miR binding to the probe. The analyte binding displaces a reporter strand to induce a signal change. Although the reporter protects part of the probe from off-analytes, the toehold region is not protected. If the probe’s toehold sequence matches both the analyte and off-analyte false signals well be seen. To mitigate this problem, the sensor’s molecular configuration can be designed so the location of the unprotected toehold region contains bases that are fully complementary to the analyte, but not the off-analyte. The mismatched base pairs between the probe’s toehold and the off-analyte will weaken the binding interaction. The weaker binding will discourage the displacement of the reporter. Here we will compare the effect of altering the location of the toehold region on the probe to determine how much the selectivity for the analyte of interest can be increased. We will discuss the differences in selectivity and sensitivity for a miR-146a-5p biosensor in the presence of different naturally occurring mismatch sequences.

Keywords: Bioanalytical, Biosensors, Fluorescence, Nucleic Acids
Application Code: Bioanalytical
Methodology Code: Sensors
Small thiols such as cysteine play key roles in biological function. Abnormal concentrations of cysteine are observed in patients with diseases such as Alzheimer’s, Parkinson’s, cystinuria, and cystinosis. Sensors with high cell-permeability and low interference are needed for real-time measurement of intracellular thiols and disulfides since the ratio provides insight into the redox network inside cells.

Radioisotopes are used in bioanalytical measurements as they provide the lowest perturbation on analyte properties, such as binding kinetics. 35S isotope has higher decay energy than 3H and the resulting beta particles travel a longer distance (ca. 300 μ in water), making 35S a better tracer for sensitive detection of sulfur-containing analytes.

In this research, polystyrene-silica core-shell nanoparticles doped with reporter fluorophores 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 successfully utilized to quantify cysteine at sub-nM concentration. The signal enhancement by specific binding of 35S-cysteine to these nanoparticles was one order of magnitude in a separation free assay. The extent of binding depends on the ratio of cysteine to cystine which is a function of pH, oxidation state of the compounds, and the presence of thiol-reactive agents. We applied nanoSPA sensors to measure thiols and disulfides, by protecting the thiols and reducing disulfides to more thiols. We also indirectly measured thiol-reactive agents such as HNO, which shows promising pharmaceutical properties.

**Keywords:** Bioanalytical, Biosensors, Quantitative, Sulfur
Pi-Pi Stacking of Pyrene Carboxylic Acid with Carboxylated Multiwalled Carbon Nanotubes for Sensitivity Enhancement of Clinical Immunosensors

The detection of biomolecules provides a significant advancement in the fields of healthcare, safety, environment, and others. Clinical immunosensors are associated with earlier detection and monitoring of diseases, and the way forward is to have these sensors ultra-sensitive and ultra-fast. Given the large conductive surface area and ease of functionalization, carbon nanotubes based sensors are prone to be the next generation of ultra-sensitive detecting systems. With this in mind, we have designed a serum insulin immunosensor with covalent and noncovalent carboxylations of carbon nanotubes. The stacking of 1-pyrenebutyric acid (Py-COOH) with carboxylated multiwalled carbon nanotubes (MWNT-COOH) on gold screen printed electrodes provides for the covalent immobilization of monoclonal insulin-antibody (Ab(surface)) and detection of serum insulin captured by horseradish peroxidase-labeled second insulin-antibody (Ab[sub]HRP[/sub]) attached to magnetite nanoparticles. Linear amperometric responses were recorded for increasing concentrations of serum insulin when a solution of 1 mM hydroquinone and 0.5 mM H2O2 in phosphate buffer saline (pH 7.4) was delivered to the sensor surface at an applied constant potential of -0.1 V. This strategy provides a 2.5-fold greater sensitivity versus the use of MWNT-COOH alone for serum insulin measurements, and a low detection limit of 2 pM insulin spiked in 5% human serum. In addition to electrochemical detection, this methodology is applicable to optical and spectral based detection methods.

Acknowledgement
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Keywords: Bioanalytical, Biosensors, Electrodes, Immunoassay
Application Code: Bioanalytical
Methodology Code: Sensors
A Novel Bioassay Platform Using Silica Core Liposome Shell Microparticles for Ligand Discovery

Multiple disease states are caused by dysregulation of biochemical pathways through ligand-receptor interactions. Discovery of ligands that target transmembrane proteins is limited to platforms that support protein function. There is a need for rapid and highly specific assay platforms for identifying novel ligand receptor interactions while minimizing crosstalk and non-specific binding. To address this need, we have developed a novel microparticle architecture that utilizes silica core particle that is functionalized with receptors within liposomes. This particle architecture is then used to perform pulldown assays in complex solutions with subsequent analysis by electrospray ionization (ESI) or matrix-assisted laser desorption ionization (MALDI) mass spectrometry. As a proof-of-concept, recovery of a fluorescent serotonin antagonist (NAN-190 analog) via binding to 5-HT1A receptors within CHO-K1 cell membranes was evaluated. Native CHO-K1 cell membrane fractions and those overexpressing the 5-HT1A receptor were isolated through homogenization and centrifugation, and were extruded to form 200 nm vesicles. The vesicles were then immobilized to the particle surface to yield silica core-cell membrane vesicle shell particles. NAN-190 was incubated with these particles, and centrifugation was used to pull down and wash the particles to reduce nonspecific binding. Using the liposome shell microparticles with overexpressed 5-HT1A receptors allowed for 2x enhancement in NAN-190 binding. To simulate a complex biological environment, the NAN-190 incubation was done in the presence of serum. NAN-190 was able to specifically bind to the 5-HT1A receptors in this complex biological matrix. This platform shows great promise for use as a high throughput drug screening platform, as a preconcentration method for diagnostic biomarkers in a hospital setting and as a new way to discover other novel binding moieties to transmembrane proteins.
Bipolar electrodes (BPE) are electrically floating metallic elements placed in electrified fluids that enable the coupling of anodic and cathodic redox reactions at the opposite ends. One particularly promising application allows electron transfer reactions at one end of a closed BPE to be read out optically at the other end. Herein, two kinds of detectors based on closed BPE with separated analytical and reporting part has been fabricated and characterized. The first geometry of closed BPE consisted of separated analytical and reporting microchannels integrated by bipolar interdigitated electrode arrays (IDEAs). Reactions amplified by redox cycling in analytical microchannel could be monitored by a fluorogenic reaction coupled in a separated reporting channel. These bipolar IDEAs also allowed to capture the spatial distribution of electrochemically active species through in situ fluorescence imaging. In order to simplify the configuration of closed BPE and detection process, a BPE-enabled device based on two thin-layer-cells was fabricated to couple an analytical reaction in one cell with a colorimetric reaction in the reporting cell. The electrochemically induced color change was determined by measuring its absorbance via a CCD camera or was further simplified through smartphone-based detection and RGB analysis. In addition, the applied potentials can be controlled either conventionally with a potentiostat, or with AA batteries. The combination of battery-powered operation of a compact closed-BPE dual cell configuration with a smartphone camera yields a simple, inexpensive, field-deployable electrochemical sensor.

Keywords: Analysis, Electrochemistry, Lab-on-a-Chip/Microfluidics, Sensors
Application Code: Bioanalytical
Methodology Code: Electrochemistry
Trace element analysis in high matrix samples such as herbal supplements presents a challenge due to their complex and variable composition. Although ICPMS is currently the most commonly used technique for elemental analysis, the complex matrix of herbal supplements results in formation of polyatomic interferences which can impair its analytical performance. However, isotopic dilution mass spectrometric (IDMS) method is known for its accuracy, precision and sensitivity particularly for the determination of trace level analytes in complex matrices. It is routinely used by metrological entities for the certification of reference materials. Combining the analytical characteristics of IDMS with kinetic energy discrimination ability of ICPMS and the ease of sample preparation offered by online isotopic OID approach presents a method that should facilitate reliable measurement of trace elements herbal supplements. In this work, we validated and applied an online isotopic dilution ICPMS method for trace element analysis of 18 herbal supplements (6 types, 3 brands). Results on the analytical performance of the method, daily exposure to trace elements via consumption of these supplements, comparison to daily recommended intakes intake (EAR, AI, and RDA), UL, and the maximum contaminant levels in food will be presented. Multivariate statistical approaches such as principal component and hierarchical cluster analyses will be used to understand the similarities or dissimilarities among the supplement brands.

Keywords: Elemental Analysis, Food Safety, ICP-MS
Application Code: Food Safety
Methodology Code: Atomic Spectroscopy/Elemental Analysis
Considerations for the Analysis of Cremated Remains by Inductively Coupled Plasma-Atomic Emission Spectrometry

In 2002, bodies were discovered on the grounds of the Tri-State Crematorium in Noble, GA. After investigation by the authorities, it was determined that the owner improperly disposed of hundreds of individuals and provided families instead with concrete dust. Since that time, a number of analytical methods have been investigated to establish the authenticity of human cremated remains. In this study, two certified reference materials, bone ash powder (NIST 1400) and Portland cement (NIST 634a), were used to design microwave digestion protocols and the resulting solutions were analyzed with Inductively Coupled Plasma-Atomic Emission Spectrometry. Using these methods, authentic human cremated remains, animal cremated remains, non-cremated remains (e.g. cement, grout), and adulterated cremated remains were examined. Elemental responses from the digested materials were successfully utilized to distinguish cremated remains from adulterated and non-cremated remains materials.
Studies have established the significance of trace metal determination in seafoods. This includes the need of quality data for safety (contamination), provenance, health risk assessments and regulation enforcement. Diop et. al (1) observed high concentrations of arsenic and site related variation in trace element content of shrimps from Senegalese coastal waters. Carter et.al. found high concentrations of arsenic and cadmium in Australian prawns. These authors also used trace metal profiles (with stable isotope data) to distinguish Australian prawns from neighboring Asian countries. Ortea and Galaro (3) utilized multi-element (As, Cd, and Pb) composition to distinguish between wild and farmed shrimps. Determination of trace elements in biological tissues with the inductively coupled plasma mass spectrometry (ICPMS) is not trivial. Their chemical matrix contributes to the formation of polyatomic interferences that can adversely affect the analytical performance of this technique. In this study, an online isotopic analysis (OIDA) method based on ICPMS was validated and applied for trace element determination in shrimps. The validation results, estimated daily intake of trace metals via consumption of shrimps analyzed, evaluation of safety by comparison to maximum contaminant levels and the daily recommended intake values are presented.

Abstract Text
A milk contains various mineral elements and protein. Laser induced breakdown spectroscopy (LIBS) technique is used to compare the various types of commercial non-dairy and dairy milk powder products. LIBS was investigated for the determination of the elemental composition of soy, rice, and almond milk powders and different percentages of dairy milk fat. The analysis is performed using radiative transitions from the atomic and molecular emissions. The atomic emission from Ca, P, Zn, K, Na, and Mg lines and the molecular emissions from $\text{C}_2$ and CN bands observed in LIBS spectra of non-dairy and dairy milk were compared. In addition, protein and fat level in milks can be determined using molecular emissions such as CN band. LIBS spectra were collected by varying various parameters, such as laser energy, gate delay, and gate width to optimize the LIBS signals.

Keywords: Atomic Spectroscopy, Food Science, Molecular Spectroscopy, Plasma
Application Code: Food Science
Methodology Code: Atomic Spectroscopy/Elemental Analysis
**Session Title**  
Atomic Spectroscopy - Environmental, Food, Fuels, Metals

**Abstract Title**  
Total Organic Carbon (TOC) Analysis of Soil and Rock Comparing Various Elemental Analysis Techniques

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**Abstract Text**

Total Organic Carbon (TOC) in soil and rock is a common analysis used for determining locations of natural hydrocarbon fossil fuel deposits. Direct measurement of TOC is difficult due to the unique properties of soil and rock and must be performed using a temperature dependent differentiation method. TOC determination is most commonly performed indirectly by acid treatment of the sample to effervesce the carbon dioxide from the inorganic carbonate species. Several documented methods, including ISO 10694, recommend the use of Hydrochloric acid for the removal of carbonates as it is strong enough to react with the carbonates, but does not react excessively with organic carbon. This method is sometimes referred to as Non-Carbonate Carbon and is used to closely estimate TOC.

Total carbon combustion instruments use either a high temperature resistance furnace or a high temperature induction furnace to achieve complete decomposition of the sample. The organic carbon is oxidized by the pure oxygen environment and converted to CO2. The gas is swept through the instrument reagents and carried to the infrared detectors where the infrared absorbance of CO2 is measured and converted to a quantifiable concentration based on initial sample mass.

This poster presentation will cover the comparison of TOC determination on various total carbon analysis techniques with or without the use of acid treatment to remove the inorganic carbon species. This comparison will include samples that are hydrophilic and hydrophobic to the acid treatment process. Data will be examined that includes soil reference materials and rock samples.

**Keywords:**  
Elemental Analysis, Fuels\Energy\Petrochemical, Soil, Total Organic Carbon

**Application Code:**  
Food Safety

**Methodology Code:**  
Atomic Spectroscopy/Elemental Analysis
Limit of detection is a key characteristic of any ICP method as it defines the “minimum concentration of an analyte (substance) that can be measured and reported with a 99% confidence that the analyte concentration is greater than zero” (EPA, 40 CFR Part 136). It is calculated as a multiple (student’s t-value) of the standard deviation of a repeated analysis when the concentration reaches zero. Detection limits depend on multiple factors in the process of sample preparation and analysis. An important factor that can enhance or decrease detection limits is the time of acquisition of the signal. The longer a signal is observed, the more variations in signal intensity are compensated for and the better the detection limits are. However, the improvement of detection limits in this case comes along with an increase in analysis time, often unacceptable for routine applications. The user has to find a compromise between detection limits of the method and speed of analysis. This poster shows a comparison of detection limits achieved with different acquisition times and gives an outlook on ways to improve detection limits without compromising speed as well as ways of decreasing analysis time without compromising detection limits. As an example, optimum parameters for a key application of ICP-OES analysis is presented.
Radio frequency discharge (RFD) at low pressure has been well established and known for the elemental determination in solid samples, but few application of this mode were investigated at atmospheric pressure. Herein, pulsed RFD employed as a direct solid sampling technique was developed and investigated in mixture of N2 and Ar ambient at atmospheric pressure. Capability of the pulsed RFD for directly sampling nonconductive solids was demonstrated by the formed crater with the diameter of 80 μm and depth of 50 μm on sample surface with an averaged input power of 65 W after discharge for 1 min. Optical emission results show that the type of this discharge belongs to the conventional atmospheric-pressure RF discharge. After combining the pulsed RFD with inductively coupled plasma mass spectrometry (ICPMS), capability of quantitative analysis was demonstrated by the use of two types of standard oxide samples with relative standard deviation (RSD) of ~ 20%. For most elements, good calibration linearity and limits of detection (LODs) in range of 10-8 ~ 10-9 g/g were achieved. All these results suggest that this technique could be an inexpensive yet effective alternative to the popular laser ablation technique.

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The faith of carbon dioxide after sequestration is of prime interest for the development of a sustainable carbon sequestration technology. In response to this worry, a constant monitoring of the injected site is required. Subsurface fluid sample and tracer analysis have been used as CO2 monitoring techniques. These techniques give precision on the alkalinity, pH and real-time fluid gas compositions which are highly effective parameters to track the CO2 plume. However, sampling of subsurface is difficult because fluid mixtures such as CO2, brine, and hydrocarbons density-separate in the wellbore, temperature and solubility relationships change, and preserving samples at in-situ temperature and pressure conditions is a major challenge. Also, these methods cannot be used as a standalone technology and analysis of major and minor elements is carried in laboratory with techniques such as mass spectrometry. In effect, little attention has been accorded to the chemical analysis of trace elements in such environments. But by monitoring the variation of these trace elements in the presence of dissolved CO2 at high pressure, one can predict the degradation of the of the storage site. Since monitoring requires in-situ technology, the optical nature of the laser-induced breakdown spectroscopy makes it an ideal alternative.
The nutritional content of food is composed of a variety of components, including vitamins, minerals, and organic and biological species. Many of these components are endemic to the food itself but are also influenced by environmental factors, including the water and soil in which the food is grown. To understand the mineral contribution to the health of foods, it is important to determine their mineral content, as well as that of the soil where plants are grown. Since the number of analytes is relatively small (generally less than 10) and the concentrations high, inductively coupled plasma optical emission spectroscopy (ICP-OES) is an ideal technique for these analyses.

This work will focus on the determination of mineral elements in soil and food, in the form of fruit juice and milk, using microwave sample preparation and ICP-OES analysis.

**Keywords:** Agricultural, Environmental Analysis, ICP, Soil

**Application Code:** Environmental

**Methodology Code:** Atomic Spectroscopy/Elemental Analysis
The use of cerium dioxide and zinc oxide nanoparticles (NPs) is increasing each year as new applications are developed. While these products can provide enhanced functionality for commercial products, they may pose harm to human, animal, plant, and aquatic life. Drinking water is one of the most direct sources for human consumption of NPs; thus, evaluating impact of drinking water treatments on NPs present in source water is imperative. In our work, single particle (SP)-ICP-MS methods were developed to detect Ce- and Zn-NPs as commonly produced NP oxides (CeO$_2$ and ZnO, respectively) in surface water and drinking water. River water was collected and water treatment procedures were conducted to determine NP removal by the following sequence: 1) lime softening, 2) various coagulation processes (alum, ferric chloride, and ferric sulfate) with and without powdered activated carbon sorption, and 3) disinfection by free chlorine. The same procedures were conducted in the same matrix with the addition of 30-50 nm CeO$_2$ NPs and/or 80-200nm ZnO NPs. The results indicated that lime softening to pH 11 could remove more than 99% of CeO$_2$ NPs (present in the water or added engineered) and up to 53% of ZnO (present in the water or engineered). When alum coagulation immediately followed lime softening, ZnO NP removal increased up to 73% of the initial particle concentration while sequential lime softening had minimal effects on ZnO NPs. In a separate study, river water was collected and NPs were added to evaluate the efficacy of three types of coagulants under Zone 2 and Zone 4 treatment conditions. Alum, ferric chloride, and ferric sulfate were selected based on their prevalent use in drinking water treatment. Zone 2 treatments with each coagulant resulted in 90-99% removal of the selected NPs. Zone 4 treatments varied among the coagulant used: alum removed 92-98%, ferric chloride removed 98-99%, and ferric sulfate removed 97-99% of the initial particle concentration of CeO$_2$ and ZnO.
During the last decade, the production and use of engineered nanomaterials (ENMs) have experienced a drastic increase, resulting in a potential risk of their release into the environment. Therefore, the study of their impact on the environment becomes crucial. The appropriate ecological risk assessment and management of ENMs in the environment requires quantitative measurements of both exposure and effects that should, ideally, be performed by in situ analysis and give physicochemical characterization. However, most analytical techniques (TEM, SEM, DLS…) are not suitable for environmental matrices since nanoparticle concentrations are very low.

Alternatively, single particle inductively coupled plasma mass spectrometry (SP-ICP-MS) has been found to be a promising technique for detecting and characterizing metal nanoparticles at very low concentrations. SP-ICP-MS is fast and efficient and can provide more information than other currently available techniques. It can lead to the determination of particle size, size distribution, particle number concentration, and the concentration of dissolved metal. Moreover, it can distinguish between particles of different elements.

The aim of this work is to investigate the efficiency of SP-ICP-MS for the detection and characterization of metal nanoparticles in environmental matrices where they can be involved in various physicochemical processes.
Gunshot Residues (GSR) contains micro and nanoparticles resulting from the discharge of firearms. GSR originates from the primer and propellant, as well as from the metallic components of the ammunition and firearm. The research presented herein considers a typical particle composition containing antimony (Sb), barium (Ba) and lead (Pb). Scanning Electron Microscopy (SEM) with energy-dispersive X-ray (EDX) spectroscopy is the state of the art GSR analysis method. However, the setup and manual confirmation of results is tedious and time-consuming. The automated search of one blank stub can take 2 to 6 hours, depending on the instrument and chosen parameters; and the duration of analysis could increase greatly if a sample contains a large number of detected particles.

This research focus is on the use of single particle Inductively Coupled Plasma Mass Spectrometry (SP-ICP-MS) to analyze GSR particles. With the availability of new analytical instruments, it has been possible to identify and characterize GSR nanoparticles in a given sample in 10 minutes. With the aid of an auto sampler over 100 samples can be analyzed per day, independent of the number of particles in the sample. Another advantage of the technique is the possibility to have fully automatized post processing. Swab samples collected from shooters’ hands were sonicated in 10 mL water and analyzed with this technique resulting in more than 600 particles per mL. The low cost of analysis and less time-consuming sample preparation and analysis makes this new approach a promising procedure for GSR identification and characterization.
Mercury is a naturally occurring element found in crude oil, natural gas, shale gas, and condensates, and it is commonly found at levels ranging from low part per billion (ppb) to low part per million (ppm). For refineries and gas processing plants, mercury is a very troublesome hydrocarbon component to deal with.

Mercury’s effects on refineries and gas processing plants are numerous, including the poisoning of hydrogenation catalysts, amalgamation with metal alloys, liquid metal embrittlement, and exposure risks for employees. Altogether, these issues can introduce enormous financial and employee health costs for companies that use these hydrocarbons to produce so many of the products that we use on a daily basis. For this reason, it is extremely critical that accurate mercury analysis is performed prior to handling these hydrocarbon streams.

The standardized methods for liquid hydrocarbons (UOP 938-10) and gaseous hydrocarbons (ASTM D-6350 & ISO 6978-2) exist for this very purpose of ensuring that the mercury concentrations in liquid and gaseous hydrocarbon streams are accurately quantified and not underestimated.

In this presentation, mercury concentrations in multiple liquid hydrocarbons will be measured using the Model PE-1000 Mercury Analyzer from Nippon Instruments Corporation (NIC). The samples will also be measured using an alternative technique to illustrate effectiveness and accuracy.

Keywords: Elemental Analysis, Fuels\Energy\Petrochemical, Gasoline, Mercury
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Atomic Spectroscopy/Elemental Analysis
The need for portable, hand-held mercury monitors that accurately measure mercury levels without interferences or false-positives is very important. OSHA and other public health agencies have placed limits on amount of mercury someone can be exposed to over time in order to protect the health of workers that may come in contact with mercury.

There are limits for total exposure over a period of time, and there are also absolute limits that require the workers to use different forms of personal protective equipment (PPE) when these limits are exceeded. Many of the areas that require such monitoring contain gases from industrial processes that may interfere or effect the accuracy of the mercury monitor. There are various detection methods currently being used in mercury monitors, and some of these methods are more likely to be effected by changes in the ambient air being monitored. When it comes to worker safety, the accuracy of the handheld mercury monitor is crucial.

In this presentation, a comparative study will be presented showing the effectiveness and accuracy of atomic fluorescence spectroscopy versus atomic absorption as used by the Model EMP-2 Mercury Monitor from Nippon Instruments Corporation (NIC). Multiple gas types will be surveyed.

**Abstract Text**
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**Keywords:** Atomic Absorption, Industrial Hygiene, Mercury, Portable Instruments

**Application Code:** Industrial Hygiene

**Methodology Code:** Atomic Spectroscopy/Elemental Analysis
Mercury is broadly dispersed through the Earth’s environment due to both natural processes, such as volcanic activity, as well as anthropogenic processes, such as coal fired utilities and mining. The bio-accumulation effect of mercury in the food cycle of living organisms means that even trace levels of mercury in the environment can compound and magnify into levels that can have toxic neurological effects on humans.

EPA Methods such as 1631E and 245.7 recommend the use of atomic fluorescence spectroscopy to achieve quantitation limits for mercury as low as 0.5ppt (1631E) or 5ppt (245.7). At such trace levels, the entire methodology used for mercury measurement from collection through to measurement is critically important. The cleanliness of both those collecting the samples and the environment for the analysis must be considered in order to achieve accurate mercury measurements at these levels.

In this presentation, mercury concentrations in multiple wastewater samples is measured using the Model RA-4300FG+ Mercury Analyzer from Nippon Instruments Corporation (NIC). The techniques used and results achieved will be discussed in detail.
Using photochemistry and mass action principles, our research has developed a method for the quantitative detection of cyanide within a blood sample. Due to the high binding affinity of cyanide to iron, direct measurement of cyanide is difficult, and because of the high amount of iron within blood, indirect measurement also proves difficult to achieve. The use of ultraviolet-visible radiation to dissociate cyanide from iron allows for binding of free cyanide to silver solid to form silver-cyanide metal complexes, which are then accurately detected using flame atomic absorption spectroscopy. The efficiency of this analytical method avoids the need for additional reagents, reducing sample contamination, and is carried out at elevated pH to improve safety and minimize sample loss through acidic off-gassing. When this procedure is coupled with AAS, this method provides a methodology that is cost-effective, uses small sample volume, minimal sample preparation, and nominal analyst training for accurate quantitation of cyanide within blood.
Sample introduction systems play an important role in daily laboratory work. They help to streamline daily laboratory work flow to improve productivity. Complete sample introduction systems, including autosampler, autodilution and intelligent dilution solutions provide a single, simple integrated workflow, eliminating manual dilution in both prescriptive and intelligent, fully automated, analyses. Eliminating manual intervention increases productivity, prevents re-runs and reduces cost of ownership.

In this poster U.S. EPA method 200.7 analysis of drinking and waste waters, using inductively coupled plasma optical emission spectroscopy with an autodilution system is described. This paper presents the results from the analysis of water samples with all necessary quality control procedures based on the requirements of U.S. EPA method 200.7, including manually spiked samples to show the different capabilities of sample introduction solutions.

Keywords: Environmental/Water, ICP, Sample Handling/Automation, Sample Introduction
Application Code: High-Throughput Chemical Analysis
Methodology Code: Atomic Spectroscopy/Elemental Analysis
Nitrogen and Carbon determination by combustion analysis is very commonly used for soils, plants and leaves analysis because these elements provides important information for their characterization in agricultural and environmental research. However, also sulfur content is sometimes required. A deficiency of sulfur has an influence in the growth of vegetables, in the quality of proteins, synthesis of vitamins and also has an effect on the formation of chlorophyll. Additionally sulfur in leaves can be considered an indicator of pollution. As the demand for improved sample throughput, reduction of operational costs and minimization of human errors has increased dramatically, a simple and automated technique which allows fast NC analysis and sulfur when it is requested, is the key for modern laboratories. The FlashSmart Elemental Analyzer is equipped with two totally independent furnaces allowing the installation of two analytical circuits which are used alternatively for NC or Sulfur analysis and is completely automatic through the MultiValve Control (MVC) Module reducing the timing to pass from one to another configuration. This paper presents NC and Sulfur data of several soils and plants to show the repeatability, accuracy and precision obtained.

Keywords: Elemental Analysis, Laboratory Automation, Soil
Application Code: Agriculture
Methodology Code: Atomic Spectroscopy/Elemental Analysis
Recently, laboratories have suffered from increasing analytical costs due to worldwide reduced availability and higher market prices of helium. Elemental analyzers, including the FlashSmart Analyzer, use helium as a carrier and reference gas during periods of sample analyses and instrument Stand-By. There is therefore a demand for reduced helium consumption or the use of an alternative gas, such as argon, which is more readily available and at lower cost compared with helium. This paper presents several workflow options to reduce helium consumption using the FlashSmart Elemental Analyzer including the use of a new device, the MultiValve Control (MVC) Module, which switch automatically from helium to nitrogen or argon gas, when the instrument is not used overnight, or on weekends, or for a prolonged period of time. Also the use of argon as carrier gas is discussed. With these options it is possible to increase the system productivity and reduce the cost per analysis.

Keywords: Elemental Analysis, Laboratory Automation, Optimization
Application Code: Laboratory Management
Methodology Code: Atomic Spectroscopy/Elemental Analysis
Carbon, Nitrogen, Hydrogen, Sulfur determination by combustion analysis, and Oxygen determination by pyrolysis are fundamental for quality control and R&D purposes in all application fields. The use of an accurate and automatic analytical technique which allows the fast analysis with an excellent reproducibility is required. For this reason a dedicated device, the MultiValve Control (MVC) Module has been developed for the FlashSmart Elemental Analyzer. 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 MVC Module controlled by the EagerSmart Data Handling Software. The MVC Module also allows to reduce the helium carrier gas consumption by switching from helium to nitrogen or argon gas, when the instrument is not used overnight, or on weekends, or for a prolonged period of time. In this way the FlashSmart Elemental Analyzer coupled with the MVC Module copes effortlessly with the wide array of laboratory requirements such as accuracy, day to day reproducibility, high sample throughput and lower cost per analysis. This paper presents data on CHNS/O determination to show the performance of the system.
The purity of a metal plays an important role in its functionality in the final products in which it is used, with impurities generally causing failure modes. Therefore, it is important to accurately measure trace impurity levels to properly characterize metal prior to its inclusion in the production process.

ICP-MS is the best technique to measure trace levels of metals, but high matrix levels must be diluted prior to entering the instrument to avoid instrumental drift and/or contamination. When measuring trace levels, sample dilution should be minimized, as higher dilution factors affect the lowest levels which can be accurately measured.

An alternate approach to dilution involves sending small aliquots of undiluted sample directly into the ICP-MS. The lack of dilution means that lower levels can be accurately measured, but the small sample size results in a short measurement window, which may compromise accuracy. However, by using an ICP-MS capable of fast measurements, accuracy and precision are not limited.

This work will discuss the analysis of high-purity metals using ICP-MS with FAST-FIAS sample introduction: the FAST FIAS introduce undiluted aliquots of sample to the ICP-MS which performs the analysis.
Current ICP-MS instruments due to improvement in the ion path design and the introduction system can analyze routinely up to 0.1%-0.5% total dissolved solids (TDS). However, samples with higher TDS have to be diluted prior to the analysis. This dilution helps to minimize matrix suppression and reduce deposition of salts on the ICP-MS cones during long analytical runs. It can be achieved by a manual off-line dilution, or an automatic on-line liquid dilution, or by a direct gas/aerosol dilution.

The PerkinElmer’s All Matrix Solution (AMS) gas dilution system for the NexION’s and ELAN’s ICP-MS instruments allows all types of matrices with high total dissolved solids to be analyzed without liquid dilution prior to analysis. The AMS is an elegant system that allows ICP-MS laboratories to avoid an extra manual off-line operation with its inherent risk for potential error and contamination. The system is simple to operate, requiring no special instrument optimization for maximum productivity.

It will be shown that the AMS, in combination with a well design system introduction system, is a perfect technique for the analysis of diverse, high TDS types of matrices without an extra sample preparation. Results of CRM analysis and the stability of the AMS dilution will be shown and discussed.

**Abstract Text**

Current ICP-MS instruments due to improvement in the ion path design and the introduction system can analyze routinely up to 0.1%-0.5% total dissolved solids (TDS). However, samples with higher TDS have to be diluted prior to the analysis. This dilution helps to minimize matrix suppression and reduce deposition of salts on the ICP-MS cones during long analytical runs. It can be achieved by a manual off-line dilution, or an automatic on-line liquid dilution, or by a direct gas/aerosol dilution.

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**Keywords:** ICP-MS

**Application Code:** General Interest

**Methodology Code:** Atomic Spectroscopy/Elemental Analysis
The ability to measure elements in oil is of great importance to a variety of industries, ranging from petrochemical companies assessing the quality of their products to operators of heavy equipment, with each having different needs and requirements. ICP-OES is an ideal technique for these analyses due to its matrix tolerance, ability to handle organics, multi-element capability and high sample throughput analysis. Because of these features, ASTM has developed methods centered on the use of ICP. This work will discuss various ICP-OES analyses of oil and its compliance to ASTM methods such as D4951 and D5185.

Keywords: ICP, Metals, Petrochemical
Application Code: Other
Methodology Code: Atomic Spectroscopy/Elemental Analysis
Overcoming Difficult Interferences in Metallurgical Matrices with Reaction Mode ICP-MS

When analyzing samples via ICP-MS, interferences on the analytes of interest are an issue that must be dealt with. As ICP-MS instrumentation has developed throughout the years, a number of techniques have been implemented to deal with interferences, ranging from mathematical correction equations to varying the resolution of the instrument to chemical reactions. Interferences can originate from several sources, including the sample solvent, contaminants in the argon gas used to create the plasma, and the sample matrix itself. These interferences can take many forms, including matrix-oxides, matrix-argides, and double charged species of the matrix (to name a few), all which originate in the plasma.

Analyzing metallurgical samples presents unique challenges because the matrix species are high concentrations of the metal(s), which results in high levels of specific interferences, mostly metal-oxides, metal-argides, and doubly-charged species of the metal. Many of these challenging interferences can be removed by selecting appropriate reaction chemistry in an ICP-MS equipped with reaction mode capabilities.

This work will focus on the elimination of challenging interferences in metallurgical matrices with ICP-MS.

Keywords: ICP-MS, Metals, Trace Analysis
Application Code: Other
Methodology Code: Atomic Spectroscopy/Elemental Analysis
Although the trend in elemental analysis is towards measuring lower concentrations, there are still plenty of analyses which require accurate measurements of high concentrations. In these situations, ICP-OES is an ideal technique due to its high matrix tolerance and multi-elemental capability. The most common ways to extend the dynamic range with ICP-OES are using radial viewing and picking wavelengths with less intensity. However, another option is running in attenuation mode which selectively suppresses the signal for a given wavelength without affecting others. Attenuation mode is a simple way to measure higher levels without having to evaluate other wavelengths for potential interferences, switching views, or diluting the samples, which can affect sample homogeneity, pH, and accuracy. As a result, attenuation mode is especially useful when measuring majors and minors in the same method. This work will discuss attenuation mode and show examples of its use in ICP-OES analyses.

Keywords: Elemental Analysis, ICP, Instrumentation, Metals
Application Code: Other
Methodology Code: Atomic Spectroscopy/Elemental Analysis
# What Levels of Contaminant Detection are Needed Today in Semiconductor Industry?

For many decades, the semiconductor industry has been continuously designing new devices that are smaller, faster and consume less power than their predecessors. To maintain this trend, the critical features of these devices must become smaller and have fewer defects. Smaller diameters of chip’s features require that all liquid chemicals and solid materials used in semi processes should have less and less contaminants.

Inductively coupled plasma mass spectrometry (ICP-MS) traditionally has been an indispensable analytical tool for quality control because of its ability to rapidly determine analytes simultaneously at the ultra-trace (ng/L or lower) levels in various process materials. Traditionally, analyses are performed using conventional, hot plasma conditions in the Standard and DRC (Dynamic Reaction Cell) modes. However, a few elements with a low ionization potential measured in cold plasma and the DRC mode yield lower background due to more efficient removal of spectral interferences.

In this poster it will be shown that using hot and cold plasma conditions in the STD and the DRC modes in a single run, DLs (Detection Limit) and BECs (Background Equivalent Concentration) needed today by semi industry can be achieved. Analysis of various chemicals would be shown and discussed.

**Keywords:** Chemical, ICP-MS, Semiconductor

**Application Code:** Other

**Methodology Code:** Atomic Spectroscopy/Elemental Analysis
This presentation will describe our research efforts to evaluate the structure-retention, structure-fragmentation and other structure-property analytical relationships for a large series of substituted aminoketones related to the cathinone-type drugs. The synthesis, GC-MS, GC-MS/MS, GC-IR and related spectroscopic properties will be presented for several series of substituted cathinone derivatives.

Our research has focused on the development of regioisomer specific methods for the identification of ring substituted aminoketone compounds (cathinone derivatives). The work includes the chemical synthesis of all regioisomeric forms of selected aromatic ring substituted aminoketones; generation of analytical profiles for each compound; chromatographic studies to separate/resolve all regioisomeric aminoketones having overlapping analytical profiles, and design and validation of confirmation level methods to identify individual compounds.

Based on the structure of the unsubstituted cathinone molecule, designer modifications are possible in three distinct regions of the molecule: the aromaric ring, the alkyl side chain and the amino group. Example compounds from all three of these areas of designer modification have been reported as components of clandestine drug samples. Commercially available precursor aldehydes, alkyl halides and amines can be converted to a wide variety of cathinone-type compounds. Legal control of a specific molecule often provides the driving force for clandestine development of additional substituted cathinone designer molecules.

**Abstract Text**

**Keywords:** Analysis, Drug Discovery, Forensic Chemistry, GC-MS

**Application Code:** Drug Discovery

**Methodology Code:** Gas Chromatography/Mass Spectrometry
Antibody drugs are an important class of highly potent biopharmaceutical drugs designed for targeted therapy. They differ from conventional low molecular drugs because the antibody drugs possess the feature to work with the immune system, allowing antibody drugs have few side effects. The antibody drugs also have advantages with treatment for incurable diseases, and obtain the attention from the global pharmaceutical industry. The quality control of antibody drugs is very important, specifically the aggregates concentration. The aggregates have a potential impact on pharmacological activity and safety of antibody drugs, increasing the importance of the quality control of aggregates in the manufacturing process.

The authors have succeeded in the development of a new GFC column for analyzing antibody drug by optimizing control of the silica pore diameter and modification method. In the new GFC column, 3 μm packing material is packed into a column of 300 mm x 8 mm I.D. The sample can be analyzed within 15 minutes in case of 1.0mL/min. At the flow rate, back pressure reaches 8.0MPa, which can be used with conventional HPLC system. It's possible to use the new GFC column for the accurate separation of protein and quality control of antibody drug, because of the large pore volume.

This poster introduces the separation performance of the new GFC column, a calibration curve, the quantity limit, analysis of antibody drugs.

Keywords: Bioanalytical, Drug Discovery, Liquid Chromatography/Mass Spectroscopy
Application Code: Drug Discovery
Methodology Code: Liquid Chromatography/Mass Spectrometry
Various biologically active substances with amino or ammonium groups exist in living organisms. It is very important to analyze these compounds to research several life phenomena and diseases. Typically, reverse-phase chromatography with an ion-pair reagent is used for analyzing these compounds. In order to simplify the analysis, a new LC/MS method was studied using a new HILIC column with polyvinyl alcohol base packing material modified with carboxyl groups. An aqueous solution composed of formic acid / acetonitrile was used as the eluent. The flow rate was suitable for LC/MS analysis, and ESI-MS was used for detection. The mixed standard solution of choline, acetylcholine, and five other amine neurotransmitters (noradrenaline, adrenaline, dopamine, tyramine, and serotonin) were analyzed simultaneously. It was confirmed this method is applicable for the analysis of amino acid neurotransmitters or amino sugars. Our described LC/MS method should be more easily achieved with higher selectivity than previous methods.
Peptide mapping with LC-MS is an analytical tool utilized in lot release of Biotherapeutics. Multi-pump UHPLC systems can be configured to enable tandem analysis with two columns in parallel and thereby increase throughput. Another common assay for batch release in biopharma QC labs is LC-MS analysis of intact proteins to assess exact mass or determine other impurities using online Solid Phase Extraction followed reversed phase LC-MS. The multi-pump system can be easily configured to run also this workflow.

Methods
The Thermo Scientific™ Vanquish™ UHPLC multi-pump setup, coupled with the Thermo Scientific Q Exactive™ HF mass spectrometer was used for the tandem LC approach. A monoclonal antibody sample was digested using the Thermo Scientific SMART Digest™ kit and separated with two Thermo Scientific Acclaim™ VANQUISH™ C18 columns in tandem operation. The same multi-pump setup enabled a fully automated online SPE setup using a column cartridge for sample trapping and the Thermo Scientific MAbPac™ RP column for separation of intact biotherapeutics with conventional water/acetonitrile-based gradients. The data were acquired with Thermo Scientific Dionex™ Chromeleon™ 7.2 SR4 and the Thermo Scientific BioPharma Finder™ software 1.0 was used for data analysis.

Preliminary data
The Vanquish tandem LC-MS setup enables up to 60% higher throughput without the need of changing the actual LC gradient of validated methods and gives retention time RSD values below 0.1% for tandem as well as single column setup. The online SPE setup can be used for fully automated sample clean-up and also enables direct injection of untreated samples. For the analysis of low abundant compounds, the setup can also be used for analyte enrichment, with the possibility of high volume injections. The LC-MS system with single point Chromeleon control fulfills GMP/GLP requirements and is a turn-key solution for fully integrated and high throughput sample handling.
The debate surrounding the use of cannabinoids for medicinal purposes has been in the news for several years. Although there are at least 85 active substances identified in cannabis, many people associate the bio-botanical with the psychoactive compound tetrahydrocannabinol (THC). Recent attention has shifted to the non-psychoactive compound cannabidiol (CBD), as evidence of the medical benefits continue to grow.

Some CBD preparations are marketed as dietary supplements and claim efficacy against a range of medical conditions. For manufacturers of these preparations, it is important to monitor product stability. A change in this parameter risks consumer health and safety when toxic or unexpected degradation products form over time resulting in the delivery of a different CBD dose than expected.

Extracts prepared from cannabis products can pose a significant challenge to chromatographers due to the vast number of naturally occurring isomeric cannabinoid structural variants. When analyzing complex mixtures, it is beneficial to employ multiple strategies and utilize various analytical tools to provide a more comprehensive understanding of the components within the mixture. For example, when applying different modes of separation, one may gain additional knowledge of both the compounds present and their structural composition.

Through a preliminary forced degradation study of crystalline CBD, we demonstrate how UPLC® and UPC2® can be applied collectively to monitor solution stability. The ACQUITY UPLC H-Class instrument uses conventional reversed-phase chromatography, while the ACQUITY UPC2 provides a stereo-chemical separation of important structural isomers via convergence chromatography. When these techniques are coupled with photo diode array (PDA) and mass spectrometry (MS), the chemical and structural identity of important CBD degradation products is determined.

Keywords: Chromatography, HPLC, Natural Products, Prep Chromatography
Application Code: General Interest
Methodology Code: Liquid Chromatography
An in vitro model that mimics the in vivo vitreous redox environment has been successfully established. This system maintains desired glutathione (GSH) and glutathione disulfide (GSSG) levels by continuously pumping GSH/GSSG buffers through the sample incubation chamber. Incubating drug molecules in this system allows us to monitor product quality attribute (PQA) changes that may represent potential changes under physiological conditions. Results were used to assess criticality of PQAs. This poster will present the challenges in the development of the in vitro model and the results from two studies.

In one study, disulfide reduction of a F(ab')2 molecule containing a single disulfide linkage connecting the two Fab arms was detected. The same reaction was previously observed in a rabbit in vivo study. The reduction rate measured in the in vitro system containing 110 μM GSH closely matched the results from the in vivo study, suggesting that the established in vitro system can be used to predict in vivo behavior. Using the same system, we have also studied the trisulfide variant of another therapeutic protein product. Trisulfide, mostly found in interchain disulfide bonds, is a common modification in proteins such as antibodies, but is often under-reported. Literature reports showed that trisulfide quickly converts to disulfide in the blood stream. However, little was known about the fate of trisulfides in ocular products after intravitreal injection. Results from this study were used to assess the criticality of trisulfides in ocular products.

Keywords: Characterization, Mass Spectrometry, Pharmaceutical, Protein
Application Code: Drug Discovery
Methodology Code: New Method
PM2.5 is an important parameter for evaluation of indoor air quality. Effect of smoking cigarettes with different quality on PM2.5 concentration and distribution in indoor air was investigated by testing 32 commercial cigarette samples originated from four major tobacco agricultural areas in China. PM2.5 was measured using gravimetric method and automatic γ-ray monitor. This work quantified the instant indoor air PM2.5 concentration, and studied the relationship between PM2.5 concentration and the elapsed time after smoking, PM2.5 spatial distribution, and the effect of ventilation condition on PM2.5 concentration. Smoking induced PM2.5 was also investigated by using Micro-hyperspec VNIR (visible and near IR) imaging spectrometer to provide additional distribution and characteristics information. With consideration of data provided by cigarette manufacturers, including tartar concentration, alkaline and CO content in cigarette smoke, the effect of second-hand smoke generated by cigarettes with different quality on indoor air quality and its potential impact on public health was discussed as well.

**Keywords:** Aerosols/Particulates, Air, Environmental/Air, Monitoring

**Application Code:** Environmental

**Methodology Code:** Physical Measurements
Thermal desorbers were originally developed in response to a need to detect relatively low-boiling volatile organic compounds (VOCs) in ambient air. However, new developments have allowed this capability to be extended, with modern TD instruments being capable of monitoring compounds boiling up to C44.

Does this universality of TD also apply to the sampling technique – specifically is there a single sampling technique that can give the best method detection limits and response RSDs? The answer is no, and this poster will describe the factors that need to be considered in coming to a decision about the best sampling method (and sampling parameters) for TD-based quantification of organic vapours ranging from C2 to C44.

**Keywords:** Environmental/Air, Method Development, Sample Preparation, Thermal Desorption

**Application Code:** Environmental

**Methodology Code:** Sampling and Sample Preparation
Testing of chlorinated contaminants such as pesticides, herbicides, and organohalides has been performed for decades. Regulated by the United States by the Environmental Protection Agency, these compounds are a difficult mix to analyze due to the nature of the compounds, and are typically analyzed by dual-column GC/ECD for identification and confirmation. Standardized EPA Methods 504, 505.1, 508.1 and 8081 are particularly challenging and require targeted selectivity to resolve closely-related compounds.

In the present work, a two-column set with complimentary selectivity for chlorinated pesticides, Zebron ZB-CLPesticides-1 and -2, is used to explore the differences between and potential benefits of using hydrogen or helium as a carrier gas for challenging chlorinated contaminant separations. Methods for linear velocity optimization using the historic Golay plot are explored and applied. Optimal carrier gas and method parameters for chlorinated pesticide testing, including resolution, compound integrity, and separation efficiency, are discussed.

Keywords: Environmental Analysis, Gas Chromatography, GC Columns, Pesticides

Application Code: Environmental

Methodology Code: Gas Chromatography
Environmental Air Quality


Ambient air pollution and particulate matter cause severe health effects in the population (e.g. lung or cardiovascular diseases). Combustion emissions are considered by the WHO to be in particular harmful. In this context ship engine- and bio mass combustion-emissions are important sources of air pollution. The Virtual Helmholtz Institute HICE addresses chemical & physical properties and health effects of anthropogenic combustion emissions. We exposed human lung cells to fresh, diluted exhaust fumes from a ship engine running on heavy fuel oil (HFO) or cleaner diesel fuel (DF) as well as to wood combustion emissions. A field deployable air-liquid interface cell-exposure system in a mobile S2-biological laboratory was used. The biological effects were toxicologically and molecular-biologically characterized (multi-omics study: comprehensive transcriptomic, proteomic and metabolomic analysis). Advanced chemical and physical analysis of the exhaust aerosols was performed and correlated with the obtained biological results. Lung cell responses include inflammation and apoptosis. Surprisingly, DF ship emissions, which contain much less toxicants, induce a significantly stronger acute regulation of essential cellular pathways (e.g., mitochondrial function, intracellular transport in A549, Oeder et al, PloSone 2015) and a higher cytotoxicity (RAW macrophages, Scapariu et al, PloSone 2016) than HFO-emissions. Wood combustion emissions from different compliances induce lower acute biological impact in the exposed cells than DF and HFO ship emissions at similar exposure doses. By combining aerosol chemical and biological information, relevant compounds and factors for the observed biological effects are identified. In addition to acute effects also long-term effects, induced e.g. by analysis of carcinogenic compounds are considered.

Keywords: Aerosols/Particulates, Bioanalytical, Environmental/Air, Volatile Organic Compounds

Application Code: Environmental

Methodology Code: New Method
A validated HPLC method with UV detection for the assay and identification of Compound X was provided to Metrics Contract Services (MCS) for the release testing of Phase II clinical trial material. The method demonstrated consistent chromatography with the validating laboratory and met typical requirements for precision and linearity. A single main peak was present in the chromatogram and no impurities were observed. MCS scientists noted, during review of the chemical structure, that Compound X contained no major UV chromophores, and a very low wavelength (210 nm) was used for the analysis. Compound X is a drug substance-maleate salt. To ensure that the method with UV detection was appropriate for the identification, assay, and quantitation of impurities of Compound X, the drug substance was analyzed using an HPLC method with charged aerosol detection (CAD). Two peaks were observed in the CAD chromatogram, suggesting that the peak identified as Compound X in the original method was the maleate ion and not the active ingredient. Peak identification was further confirmed by HPLC-MS. HPLC-CAD methods for assay, identification, related substances and dissolution testing were developed and validated prior to release testing of Phase II clinical trial material, ensuring the safety and efficacy of the clinical trial material.
The second dimension (2D) separation of two-dimensional liquid chromatography (2D-LC) presents unique challenges in method development and optimization that are not typically encountered in conventional one-dimensional liquid chromatography (1D-LC). One such challenge is mitigating the dilution of analytes as they are collected from the first dimension (1D) column, injected into the 2D column, and propagated through the 2D column. Whereas in two-dimensional gas chromatography (2D-GC) thermal focusing of the analyte zone is quite straightforward because of the large enthalpy of transfer of analytes between the mobile and stationary phases, in liquid chromatography there is no operational variable that is similarly effective for promoting focusing of the analyte band. This has led a number of groups to propose and develop a variety of approaches to focusing at the 2D column inlet. These approaches mainly involve modulation of the solvent strength of the fraction of 1D effluent either before or during injection of the fraction into the 2D column. Indeed many different physical implementations of this basic concept have been described in the literature. Other approaches using solid phase adsorbents in the interface between dimensions have been described. In this presentation we will review the details of each implementation, and then compare their advantages, disadvantages, and performance characteristics as measured by 2D analysis speed, peak capacity, and most importantly detection sensitivity. These data will enable users of 2D-LC to make informed decisions about which approach to focusing at the 2D column inlet is most suitable for their application.

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

Application Code: Pharmaceutical

Methodology Code: Liquid Chromatography
A fast, simple and accurate HPLC method was developed for the quantification of PB-1503 in dosage form through on-line automatic pre-column derivatization by Agilent HPLC. This on-line automatic derivatization with Agilent HPLC has not been reported for determination of PB-1503 in literatures.

PB-1503 has no UV absorption, but has a function group primary amine, which can undergo derivatization reaction with o-phthalaldehyde (OPA). The auto sampler of HPLC draws borate buffer, sample solution and OPA reagent from separate HPLC vials, mixes them and waits 10 minutes for derivatization reaction, then draws water to terminate the reaction and injects the products. This on-line automatic derivatization method is suitable for routine analysis. The amount of reaction reagent, reaction time, reaction temperature and reaction termination are controlled by the HPLC program without additional pre-treatment on sample. Derivatization products can be injected immediately after reaction completed. Finally, a volume of 1 ul OPA reagent can reach the molar ratio (OPA: PB-1503) of 40:1 in the HPLC auto sampler, which can greatly shorten the reaction time and meet the requirement of rapid analysis.

Under alkaline conditions, reaction between the primary amine of PB-1503 and OPA can be triggered and completed (see Figure 1 for reaction pathway) in the auto sampler of Agilent HPLC and the product is separated using an Eclipse Plus C18 column (4.6 mm*150 mm, 5 um) with a gradient program at 338 nm. A disodium hydrogen phosphate buffer (pH6.0, 0.04 M) and a mixture of ACN and Methanol (50: 50, v/v) are used as the mobile phase.

The on-line automatic pre-column derivatization HPLC method was validated to be precise, accurate, reproducible and rugged for determination of PB-1503 in extended release capsule formulation. This poster presents method development, validation and results from sample analysis of various capsule formulations.
Differentiation between robustness and ruggedness (intermediate precision) maybe more of an academia topic. Detailed studies based on a factorial design to change pH-temperature-flow rate-UV wavelength, maybe good for publication. Working in a fast-paced product development environment, however, to analyze different formulas stored at various conditions, we focus on method effectiveness. Rather than making sure the method can tolerate small changes in flow rates, temperatures, or mobile phase compositions, the parameters which we consider should and can be controlled, we demand the versatility of the method and whether the method brings to us true results.

In this poster, we present some case studies. One example is to show how a gradient method can behave differently on HPLC instruments with a binary pump and with a quaternary pump. On a binary pump, there are more choices for setting up the pump operation parameters such as different compressibility for each solvent line, while with a quaternary pump, apparently not so much choice. Another example is how the differences in autosampler firmware can impact chromatographic peak carryover. Some firmware allows needle wash for more than one time, while some only allows one-time wash. The third example is to show how much sample matrix effect can have on chromatographic results. The sample responses obtained on various instruments with different method parameters can be 90% less than the standard response, which obviously causes attention. But in some cases, the difference in responses in less than 5% and maybe overlooked.

Keywords: Instrumentation, Liquid Chromatography, Pharmaceutical, Sample Preparation
Application Code: Pharmaceutical
Methodology Code: Liquid Chromatography
In pharmaceutical research, correctly targeting the delivery location and release profile of drugs is a critical challenge. Polymers are playing an increasing role in drug delivery applications as targeted controlled-release mechanisms. One potential solution is to conjugate chemotherapy drugs such as Doxorubicin (Dox) with a delivery polymer such as polyglutamate (PG). Preferential uptake of PG-Dox conjugates by cancer cells and local degradation and drug release could more effectively target cancer cells over healthy cells.

Gel-permeation chromatography (GPC) is the most widely used tool for the measurement of molecular weight and molecular weight distribution of natural and synthetic polymers. Historically, the elution volume of an unknown sample was compared with that of known standards to estimate molecular weight and distribution. However, this so-called ‘conventional calibration’ will not give accurate molecular weights for conjugated polymers like PG-Dox.

Static light scattering detectors measure the intensity of light scattered by a sample as it elutes from the column. Since the intensity of the scattered light is proportional to the sample’s molecular weight and concentration, they allow the direct measurement of the sample molecular weight independent of its elution volume. A viscosity detector can also be used as part of a GPC system to measure the parameter of intrinsic viscosity which can be combined with molecular weight data to calculate hydrodynamic radius. In combination these data overcome the limitations of conventional measurements to measure accurate molecular weight, structural changes and drug loading.

In this paper, we analysed PG, Dox, and two PG-Dox conjugates. Drug loading levels were accurately assessed and molecular weight and structural changes were also analysed. Regular use of multi-detector GPC measurements could support development and testing of these drug delivery candidates accelerating research in this area.
**Simultaneous Analysis of Dual Active Ingredients in a Pharmaceutical Formulation Containing Metformin by a Single Ion Pair UPLC Method**

Metformin, a common active ingredient for the treatment of diabetes, is not typically retained under conventional reverse phase chromatographic conditions. As a result, the analysis of dual active ingredient formulations containing Metformin typically involves the use of different chromatographic conditions for each active pharmaceutical ingredient (API) in the formulation. In this work, a single and rapid UPLC method coupled with ion-pair (IP) modifiers in the mobile phase (sulphonic acid salts) was developed to assay Metformin and another API in a fixed-dose-combination (FDC) formulation. The chromatographic conditions identified here enabled the simultaneous analysis of two APIs under 5 minutes. We also studied the effect of the alkyl chain length of the ion pair, IP/active ingredient concentration and other chromatographic parameters on APIs retention, repeatability and overall chromatographic behavior. The chromatographic conditions identified in this work demonstrate the potential of using a single method in the analysis of multi-API formulations containing difficult to retain analytes under reverse phase mode. Such methods are highly desirable in the pharmaceutical industry to increase throughput and streamline operations in analytical laboratories.

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

**Application Code:** Pharmaceutical

**Methodology Code:** Liquid Chromatography
In 2015, it was reported that more than 30% of erasers were found to contain a PAE content that exceeded the 0.1% limit of Jiangsu Province in China. Thus, it is extremely necessary to find a convenient and accurate way to detect the exact amount of PAEs existing in erasers. Graphene oxide (GO) plays an important role in solid phase extraction (SPE) for both polar and nonpolar compounds.\[sup\]1\[/sup\] Whereas, GO-based adsorbents are typically restricted by the difficult separation after treatment because of the low density and high pressure in filtration. Ionic liquids modified graphene oxide composites (GO-ILs) combine the advantages of both materials, such as high surface area and high tunability.\[sup\]2\[/sup\] Based on these theories, we prepared four new GO-IL composites (Fig. 1 (A)) to realize the accurate extraction of PAEs. It was proved that the composites could be used to extract PAEs in erasers successfully. Based on the properties, the composites were packed into a standard fixed-bed column with low pressure and showed high adsorption capacities for PAEs. And the extraction efficiencies were compared between the four GO-IL composites. Moreover, the composites maintained its extraction performances even after 11 cycles (Fig. 1 (B)), indicating the excellent reusability and desirable adsorption capacities. The results demonstrated a simple and efficient method for adsorption of PAEs from aqueous solution.

**Acknowledgement**

This work was financially supported by the National Natural Science Foundation of China (No. 21275055 and 21675053).

**References**


**Keywords:** Adsorption, HPLC Detection, Monitoring, Sample Preparation

**Application Code:** Consumer Products

**Methodology Code:** Liquid Chromatography
Proteomics and Metabolomics

Proteomic Analysis of Right-Side and Left-Side Colon Cancer Tumors

Colon cancer is a major cause of mortality, with an estimated 95,270 new cases and 49,190 deaths in 2016. Numerous studies have demonstrated that right-side colon cancer (RCC) and left-side colon cancer (LCC) exhibit distinct molecular characteristics and may require different treatments. For patients with highly advanced disease (Stage III and IV), chemotherapy and radiation therapy are standard treatments. However, the correct treatment choice for Stage II patients is less clear, and can vary according to additional risk factors. To gain a more thorough understanding of the progression of RCC and LCC, we profiled the proteomic changes that occur in primary colon cancer tissue during disease progression. We have obtained 41 Stage II colon tumor samples, including 24 RCC tumors and 17 LCC tumors for proteomic and genetic analyses. Global protein changes were analyzed using liquid chromatography-tandem mass spectrometry (LC-MS/MS). Isobaric tags for relative and absolute quantification (iTRAQ) were used for protein quantification relative to normal colon tissue. Strong cation exchange chromatography was used to fractionate samples and increase protein identifications. Analysis of protein level changes during the progression to Stage II colon cancer reveals distinct molecular signatures associated with RCC and LCC. This study also uses RTPCR to monitor transcript levels of genes postulated to be predictive of relapse in Stage II colon cancer. These findings will improve the understanding of RCC and LCC and their pathways to relapse.

Keywords: Biomedical, Clinical Chemistry, Liquid Chromatography/Mass Spectroscopy, Proteomics
Application Code: Biomedical
Methodology Code: Liquid Chromatography/Mass Spectrometry
The risk of developing ovarian cancer increases with age. Poor prognosis is associated with age, and younger patients tend to have lower tumor burden. A murine model of aging and ovarian cancer metastasis demonstrated that aged mice (20-23 months) are more susceptible to intraperitoneal metastasis than young mice (3-6 months). Gonadal adipose tissue from aged mice was found to have greater tumor burden than young mice. To investigate possible protein factors involved, we analyzed adipose tissue from the periovarian fat depots from healthy non-tumor bearing young and aged mice with high-resolution quantitative mass spectrometry-based proteomics. Adipose tissue is a poorly understood and underutilized tissue group for proteomics and requires specialized sample preparation. The presence of large amounts of lipid complicates trypsin digestion and chromatography for adipose tissue. We utilized a simple sample clean-up procedure adapted for whole tissue lysis, with centrifugation and precipitation to delipidate the samples. A gel-based proteomic procedure provided pre-fractionation and contaminant removal prior to UPLC-nESI-MS/MS analysis. Label-free quantification was performed using MaxQuant’s Label Free Quantification (LFQ) algorithms. We anticipate the analysis will provide insights into preferential colonization of aged periovarian tissue in a relevant murine model of ovarian cancer metastasis. Elucidation of protein drivers in the metastatic niche may provide targets for therapy and advances in differential care for elderly women with metastatic disease.

Abstract Text

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Keywords: Biological Samples, Liquid Chromatography/Mass Spectroscopy, Mass Spectrometry, Proteomics
Application Code: Genomics, Proteomics and Other ‘Omic
Methodology Code: Liquid Chromatography/Mass Spectrometry
Enhanced Coverage of the Molecular Content of Selected Single Cells from Rat Islets of Langerhans by Combining MALDI MS and CE-MS

Due to the clear distinction between cell types, we use pancreatic islets of Langerhans as a model to determine cell-to-cell heterogeneity and metabolic signatures of individual cells in expected homogenous cell populations. Each cell type in pancreatic islets, alpha, beta, delta, and gamma, can be identified by a specific hormone complement. While single cell MALDI MS allows us to characterize the hormones present in individual cells of an islet, hormone content has not be analyzed alongside the small molecule profile of the same pancreatic cell. Here, we perform single cell MALDI MS of dispersed cells, followed by capillary electrophoresis mass spectrometry (CE-MS) for metabolomic analysis of select cells. In this procedure, dissociated rat islets are stained with the nuclear dye Hoechst 33342, enzymatically and mechanically isolated, plated onto an ITO-coated glass slide, and examined using whole-slide fluorescence imaging to determine cell locations. Cells are then coated with 2,5-dihydroxybenzoic acid (DHB) and analyzed with microscopy-guided MALDI MS to classify each cell based on the peptide hormone content. Cells of interest are selected and their analytes extracted with a home-built liquid microjunction surface sampling probe. Extracts are concentrated and reconstituted for metabolic analysis with CE-MS. Coupling high-throughput cell classification by MALDI MS with CE-MS metabolite profiling enables the evaluation of cell-to-cell heterogeneity as a function of pancreatic cell type.

Keywords: Capillary Electrophoresis, Mass Spectrometry, Metabolomics, Metabonomics
Application Code: Genomics, Proteomics and Other 'Omics
Methodology Code: Mass Spectrometry
Abstract Text

Reversible protein phosphorylation is a key regulatory mechanism that diverse cellular processes, and deregulated phosho-signaling is often observed in many cancers. Despite the recent developments in proteomics technology, the dynamic nature and short time-scale of protein phosphorylation hinders the advancement of knowledge on the underlying regulatory mechanism of phosphorylation events.

The goal of our research is to develop a strategy that enables fast sampling and detection of key signaling phosphoproteins in complex biological samples. To achieve this goal, we developed a novel microfluidic platform which enables miniaturized and accelerated sample preparation processes, and coupled it to multiple reaction monitoring (MRM). The microfluidic chip has multiple chambers loaded with C18 and TiO2 beads, which are used for fast proteolytic digestion and phosphopeptide enrichment, respectively. The effect of different digestion and MS acquisition conditions, including digestion time and HPLC gradient, on the number of identified peptides and missed cleavages was investigated. An in-house library, containing 9738 peptides and 3109 proteins, was built from global proteomic studies of SKBR3 cells to facilitate the MRM-MS detection of phosphoproteins. The microfluidic platform enabled the detection of over 100 proteins from SKBR3 cell extracts, while the analysis time and reagent costs were reduced 10-100-fold. Preliminary experiments have demonstrated that phosphopeptides present in low copy numbers can be more reliably identified by MRM than conventional data-dependent MS acquisition protocols. Overall, the method and device have been successfully used to perform fast proteolytic digestion and phosphopeptide enrichment from simple protein mixtures, and the preliminary results demonstrate feasibility to detect cancer-related phosphoproteins from complex biological samples when coupled to MRM-MS.

This work was supported by NSF grant DBI-1255991.

Keywords: Lab-on-a-Chip/Microfluidics, Mass Spectrometry, Method Development, Proteomics

Application Code: Genomics, Proteomics and Other 'Omics

Methodology Code: Microfluidics/Lab-on-a-Chip
Laser desorption ionization (LDI) is commonly used to generate ions for analysis by mass spectrometry (MS). The most common of these techniques is matrix-assisted laser desorption ionization (MALDI), which uses small organic molecules to absorb the incident laser energy to volatilize and ionize analytes of interest. However, a number of matrix-free techniques have also been developed, which use inorganic nanostructured materials to serve this function, leading to reduced background and facilitating small molecule analysis. Among these, we have previously demonstrated that silicon nanopost arrays (NAPA) can be used for large scale analysis of metabolites and lipids from various biological samples including biofluids, organ sections, and cultured cells.

In-source fragmentation is a process wherein excess internal energy deposited during ionization causes generated ions to fragment prior to MS analysis, leading to loss of MS signal for desired compounds and/or interferences from the resultant fragment ions. This phenomenon is not unique to any specific ionization technique, and has been observed in electrospray ionization (ESI) and MALDI as well as NAPA and depends on a number of experimental factors. For example, we have previously noted the fragmentation of peptide, metabolite, and thermometer ions desorbed from NAPA at high laser fluences.

Because the extent of in-source fragmentation varies significantly across and within chemical classes and is dependent upon experimental parameters such as laser fluence, we here characterize the NAPA-LDI-MS fragmentation processes for several compound classes and experimental conditions. Tested compounds include a range of glycerolipids, amino acids, nucleosides, and others. Fragmentation in NAPA-LDI-MS is also compared to that in ESI-MS of the same compounds.
Quantitative proteomics analyses can consist of the use of heavy isotope containing compounds to tag peptides. Through isotopic labeling or isobaric tagging techniques, multiple samples are tagged, mixed, and analyzed simultaneously using mass spectrometry. Recently, our laboratory developed a technique that combines precursor isotopic and Isobaric tagging (cPILOT). cPILOT enhances sample multiplexing, reduces experimental costs and data acquisition times, and accommodates analysis of different sample types. Global cPILOT takes advantage of primary amine tagging with dimethylation at an acidic pH followed by tandem mass tagging (TMT) at a more basic pH. Overall, the goal of this project is to improve the efficiency of the labeling process and to reduce sample clean-up steps. Here we discuss a one-step tagging process through the generation of novel cPILOT reagents.

Keywords: Mass Spectrometry, Peptides
Application Code: Genomics, Proteomics and Other 'Omics
Methodology Code: Mass Spectrometry
Abstract Text
The importance of sulfur determination has grown significantly in the years in many application fields (geological, agronomy, petrochemistry, environmental, food authenticity, forensic) and many of the classical methods are no longer suitable for routine analysis. Analytical instruments based on sample combustion improve the reliability of data, without the use of hazardous chemicals. The FlashSmart Elemental Analyzer, copes effortlessly with the wide array of laboratory requirements such as accuracy, reproducibility, and high sample throughput. The Analyzer operates according to the dynamic flash combustion of the sample (modified Dumas method) and can determine the sulfur concentration in simultaneous CHNS and NCS modes, and as single sulfur analysis using the thermoconducibility detector. Additionally, trace sulfur amounts can be measured when the Analyzer is coupled with a flame photometric detector. This paper presents sulfur data of pure organic standards, reference materials and several matrices to evaluate the accuracy and precision of the data using the different configurations and detectors.

Keywords: Elemental Analysis, Quantitative, Sulfur
Application Code: Quality/QA/QC
Methodology Code: Atomic Spectroscopy/Elemental Analysis
Evaluation of DART (Direct Analysis in Real Time), Coupled to a Portable Mass Detector for Rapid Cleaning Validation

Cleaning validation is a vital part of Quality Control (QC) workflow and is defined as the process of providing documented evidence that the cleaning methods employed within a facility consistently controls potential carryover of product (including intermediates and impurities), cleaning agents and extraneous material into subsequent product to a level which is below predetermined levels. Cleaning validation is a required activity within the pharmaceutical, biological, nutritional supplement and medical device industries. From both a regulatory and industry standpoint, cleaning validation is recognised as an important activity to establish that product cross-contamination is controlled to ensure patient safety and product quality. Two analytical methods predominate cleaning validation analysis i.e HPLC/UV which is limited to chromophore containing compounds, and TOC (Total Organic Carbon) which will detect any source of carbon but is not specific and therefore anomalous results are required to be submitted for further testing i.e. LC/MS.

Here we discuss MS based strategies for cleaning validation. The Waters Acquity UPLC H-Class coupled to a Waters Acquity QDa mass detector provides a robust, sensitive and specific methodology. A more rapid and convenient analysis methodology was also evaluated using DART (IonSense, Saugus, MA, USA) coupled to a QDa, which offers a direct ambient ionisation sampling technique with little or no sample prep required. Both approaches will be evaluated for speed, efficiency, and also sensitivity to ensure sufficiently low levels of quantitation are being reached to ensure industry vessels etc are sufficiently clean.

Keywords: Chromatography, Mass Spectrometry, Pharmaceutical, Quality Control
Application Code: Quality/QA/QC
Methodology Code: Gas Chromatography/Mass Spectrometry
Influence of Different Glass Types Upon the Recovery Rates of Different Analytes in LC and LC-MS

There are a variety of possible causes for issues with the performance of LC and LC/MS systems when performing low-level quantitative analyses. While many of the causes for those issues are well understood, and therefore dealt with during normal method development procedures, the influence of the sample vessel is often not taken into consideration or underestimated at least.

Here, we present an investigation covering a range of different premium glass (low adsorption) autosampler vial types. We focused on the change in recovery rates over time of the tricyclic antidepressant Doxepin as monitored using LC/MS/MS. Doxepin was chosen as a model compound to assess the interaction of basic analytes with the interior surface of the glass vials and/or with impurities in the vessels’ material. As consistency of performance is also a concern with vials, an assessment of the variability of the measurement over time was also completed.

This investigation highlights sample vial selection as an essential consideration when performing quantitative analyses at low detectible levels. With ongoing advances in chromatography and mass spectrometry instrumentation pushing detection limits ever lower, even trace analyte loss due to interaction with the sample vessel can impact the quality and consistency of results. This is of particular importance when analyzing long automated sample sequences where consistency of results over time is essential.

Keywords: Liquid Chromatography/Mass Spectroscopy, Quality, Quality Control
Application Code: Quality/QA/QC
Methodology Code: Liquid Chromatography/Mass Spectrometry
A Novel Gas Flow Meter Which Eliminates the Need for Recalibration Returns

The ADM Flow Meter provides peace of mind for your GC analyses. These flow meters deliver an external reference for verifying gas flows and are a valuable tool for troubleshooting detector problems. The ADM Flow Meter are ideal for measuring gas streams with a changing gas composition. This is because the ADM Flow Meter measures gas volumetrically, eliminating the need to select a gas type. The ADM flow meter is also easy to maintain, due to its removable calibration cartridge. Instead of returning the meter to a third party, you simply replace the cartridge once a year to keep the meter compliant.

Keywords: Gas Chromatography, GC, Quality, Validation
Application Code: Quality/QA/QC
Methodology Code: Gas Chromatography
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<td>Primary Author</td>
<td>Kangming Ma</td>
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<td>Author</td>
<td>Eurofins QTA</td>
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**Abstract Text**

IR/NIR is a powerful analytical tools in routine quality analysis. The successful implementation of this solution has been challenged not only by the numerous commercially available instruments, the expertise to develop the methods/calibrations, but also the ultimate goal to maintain the robust and consistent performance of the methods. Eurofins QTA have introduced a patented technology which makes the IR/NIR implementation and maintenance hassle-free. QTA's methods are also listed as the ASTM and AOCS alternative methods.

**Keywords:** Infrared and Raman, Instrumentation, On-line, Spectroscopy

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

**Methodology Code:** Near Infrared
We propose a microfabricated SPR sensor chip of Kretchmann configuration integrated with functions derived from electrokinetic effect, which includes AC electroosmosis and dielectrophoresis. SPR sensor is a powerful tool that allows label-free detection of biomolecules. Improvement of its detection limit and integration of various functions on a chip are expected to broaden the potential application.

This paper reports a novel SPR sensor chip fabricated with microelectrode array to apply AC voltage, which induces circulating flow near the surface (AC electroosmosis) and the separation of biomolecules in accordance with their dielectric properties (dielectrophoresis). These effects are adjustable through the frequency of applied voltage. The circulating flow provides mixing effect, which will remove non-specific binding on the surface and enhance the binding of analyte. The dielectrophoretic separation will allow the detection of samples containing contamination. We evaluated the SPR characteristics of the chip and demonstrated the improvement of protein binding onto the chip surface using the electrokinetic effect.

SPR sensor chip with comb-shape microelectrodes are fabricated with standard UV lithography. Sensing experiments are carried out using Kretchmann-type SPR measurement system. Experimental results show the SPR characteristics of our sensor chip similar to that of standard one, suggesting the microelectrodes can work as a SPR sensor. To demonstrate the mixing effect by AC electroosmosis, we evaluated the binding of IgG molecules onto the sensor surface. The result indicates that the amount of binding increases ~2 times higher than that by a conventional chip, suggesting that successful enhancement of protein binding.

Keywords: Biosensors, Lab-on-a-Chip/Microfluidics, Microelectrode, Sensors
Application Code: Bioanalytical
Methodology Code: Sensors
Helium discharged photoionization detectors (HDPIDs) have been used in many gas chromatography (GC) systems. However, they are bulky and power intensive and cannot be used in a miniaturized GC for field applications. Here we develop a miniaturized HDPID ([micro]HDPID) for [micro]GC system, which offers low power consumption (<400 mW), low helium consumption (5.8 mL/min), rapid response (as fast as flame ionization detector - FID), quick warm-up time (~5 min), an excellent limit of detection (a few pg), a large linear dynamic range (>4 orders of magnitude), and maintenance-free operation. Furthermore, the µHDPID can be driven with a miniaturized (~5 cm × ~2.5 cm × ~2.5 cm), light (22 g), and low cost (~$2) power supply with only 1.5 VDC input. The dependence of its performance on bias voltage, auxiliary helium flow rate, carrier gas flow rate, and temperature was also systematically investigated. Finally, the [micro]HDPID was employed to detect permanent gasses and a sub-list of the EPA 8260 standard reagents that include 52 analytes. We will describe the details of fabrication and characterization of the [micro]HDPID, and show how it is implemented in [micro]GC system for in-situ, real-time, and sensitive gas analysis.
Localized Surface Plasmon Resonance shows excellent promise as next generation biosensing materials, since they provide sensitive, label-free, rapid, colorimetric detection that is amenable to on-chip devices. We have recently incorporated uniform nanoparticle arrays into microfluidic and multiplexed devices through the combination of photolithography and colloidal lithography. In addition, these uniform nanoparticle arrays can be fabricated on other substrates including flexible polymers, which make the devices less expensive and more portable. We have applied this fabrication towards rapid, point-of-care assays for various biological pathogens. These assays involve portable, miniaturized spot plates in which small sample volumes can be used to test for pathogenic species in a multiplexed manner. Combining our technology with color analyzing software available on the I-Phone enables rapid read-out in low resource settings.


Keywords: Bioanalytical, Biosensors, Biotechnology, Nanotechnology
Application Code: Bioanalytical
Methodology Code: Sensors
The classical methods of analytical chemistry, e.g. extraction and colorimetry are well-established approach allowing determination of many analytes, including metal ions, although these approaches are relatively chemical and time consuming. The possibility of improvement of these approaches, e.g. by application of nanocontainers to perform extraction and determination in is still a challenge [1].

In this work advantages and limits of application of different nanostructures – simple surfactant (e.g. cetyltrimethylammonium chloride (CTAC), sodium dodecyl sulphate (SDS)) as well as polymeric (e.g. poly(maleic anhydride-alt-1-octadecene) [2]) micelles for extraction and colorimetric determination were studied. As a model ligand 1-(2-pyridylazo)-2-naphthol was chosen. This compound was introduced to the micelles and the effect of different factors including surface charge of nanostructures, prevailing “conditional” binding constant and stability of micelles in sample environment were studied.


Keywords: Nanotechnology, UV-VIS Absorbance/Luminescence
Application Code: Other
Methodology Code: UV/VIS
UV/VIS Applications

Photometric Study of Enzymatic Reaction by Glucose Oxidase Immobilized on Iron Oxide Fine Particles

1. INTRODUCTION
Fine particles of iron oxide have been widely studied and used as magnetic materials. One of viability is to use iron oxides as magnetic carriers. Movement of magnetic particles could be controlled by external magnetic field. They may be applicable in medical science like as Drag Delivery System (DDS). In this study, magnetite and delta-FeOOH were synthesized and used to immobilize glucose oxidase on the surface as a model. The immobilization and enzymatic reaction were studied by UV-VIS spectrometer.

2. EXPERIMENT
Delta-FeOOH and magnetite were synthesized according to previous papers. The iron oxide precipitation was separated by using magnet and washed well with distilled water. Glucose oxidase was immobilized on the particles by silane coupling method, which combined enzyme to the surface by covalent bond.

3. RESULT and DISCUSSION
The immobilization was confirmed by the decrease of protein in the solution, which was measured by absorbance of 190-210nm (Lawry method). The magnetic particles were washed by distilled water up to protein free level. In buffer solution, glucose was oxidized to generate hydrogen peroxide by enzymatic reaction on the magnetic particles. The generated hydrogen peroxide was determined by photometry. The enzymatic reaction proceeded with particles modified with enzyme. On the other hand, the reaction did not occur with particles not modified. The results showed that the enzyme worked well on the surface of magnetic particles. We believe that magnetic particles modified with enzyme have potential as magnetic bio-carrier.

Keywords: Biotechnology, Enzyme Assays, Immobilization, UV-VIS Absorbance/Luminescence
Application Code: Bioanalytical
Methodology Code: UV/VIS
The lanthanide ions possess typical properties which are of industrial significance. Complexes of lanthanides have given new insights. In a similar way, kynurenic acid is having peculiar biological significance. The combination of both, in formation of novel chelates is bound to be interesting theoretically as well as industrially. In the present work, three lanthanide ions, neodymium, samarium and gadolinium were selected. Because lanthanide ions possess similar physical and also chemical properties, hence ions with atomic numbers, 60, 62 and 64 were selected. The complexes were synthesized by standard methods. These were then characterized by m.p., TLC, magnetic susceptibility, uv-visible spectroscopy, IR spectroscopy, mass spectrometry, thermal analyses etc. The structures of the complexes were ascertained with good accuracy. These complexes were subjected to anti-microbial activities by comparison with standard antibiotics. Some important chemical reactions were selected for this study. The use of small amounts of these complexes as catalysts was the next step and this study gave interesting outcome giving their utility.
**Session Title**  
UV/VIS Applications

**Abstract Title**  
Alternative Optics for Standoff Spatial Heterodyne Raman Spectroscopy

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J Chance Carter, Kevin Dudley, Patrick Barnett, S Michael Angel

**Abstract Text**

The goal of upcoming NASA planetary missions is to investigate habitability and to search for indicators of past or present life. Such missions require increasingly sophisticated means of chemical analysis especially methods that require little or no sample preparation and that can be used to measure in-situ or remotely. Thus, the development of miniature instruments that provide molecular information with no sample preparation and in a remote manner is a high NASA priority. The spatial heterodyne Raman spectrometer (SHRS) is a new type of Fourier transform (FT) Raman spectrometer that was developed in large part for planetary exploration. The SHRS is based on a dispersive interferometer, the spatial heterodyne spectrometer (SHS), with no moving parts. The SHRS has a wide input aperture and a wide field of view, which allows for very high light throughput compared to conventional dispersive, slit-based Raman spectrometers. Also the spectral resolution is not highly dependent on the input aperture size, so the SHRS footprint can be very small while still allowing high resolution Raman measurements.

The SHRS was first described by our group, recently extended to deep-UV standoff Raman, and has even been demonstrated using mm scale optics and a cell phone detector. Our current research emphasizes making truly miniature SHRS instruments of a monolithic design; making the SHRS small, sturdy and tolerant to vibrations. One goal for this design is to make the SHRS compatible with a CubeSat or SmallSat spacecraft, a type of spacecraft originally developed to increase academic involvement in space missions. CubeSats are a class of spacecraft having a standard size and form factor; the smallest unit, a 1U, measures 10x10x10 cm$^3$ and weighs less than ten kilograms, and these units can be combined up to a 12U size. Optimizing the SHRS for a SmallSat architecture requires exploring alternative optical configurations of both the interferometer and the collection optics, as well as the type of laser source. Nonconventional optics that will be discussed for this purpose include Fresnel optics for light collection, coherent fiber bundles for interferometer imaging, and diode lasers and light emitting diodes as excitation sources.

Funding for this work provided by NSF (CHE-1308211) and NASA (NNX14AI34G).

**Keywords:**  
Instrumentation, Raman Spectroscopy, Spectroscopy, Vibrational Spectroscopy

**Application Code:**  
Other

**Methodology Code:**  
Vibrational Spectroscopy
Tabletop generation of strong THz fields has enabled new linear and nonlinear spectroscopies in this little-used region of the spectrum. Dramatic new effects including THz-induced charge transfer, chemical reactions, and electroluminescence will be discussed. 2D THz spectroscopy of gas-phase molecular rotations and collective spin excitations will be illustrated. Finally, THz ESR spectra of transition metal high-spin compounds will be presented. Taken together, the results highlight the rapidly increasing range and versatility of spectroscopic methods in the THz spectral range, and in some cases they illustrate new approaches for optical control over molecular and material structure and dynamics.

Keywords: Magnetic Resonance, Material Science, Molecular Spectroscopy, Ultra Fast Spectroscopy
Application Code: Material Science
Methodology Code: Molecular Spectroscopy
The Coblentz Society/ABB - Bomem-Michelson Award

High-Throughput 2D IR Spectroscopy Platform Yields Insights into Solvent Dynamics in Solution Mixtures

In the past two decades, 2D IR spectroscopy has developed into an important tool for probing events on molecular time scales with structural resolution. For example, hydrogen bond making and breaking events occur on femtosecond time scales and can be linked to structural fluctuations in complex molecules including proteins, nucleic acids, and polymers. The use of ultrafast infrared pulses in multidimensional IR spectroscopy techniques, have afforded the timescales of structural fluctuations and solvent fluctuations to be measured and quantified. However, to fully characterize solvent dynamics, many 2D IR spectra must be collected and analyzed. For example, for a vibrational probe in a single solvent, typically more than one hundred 2D IR spectra will be collected in order gain quantitative details of the local solvent dynamics surrounding the probe. Thus, to examine the vibrational probe in solvent mixtures, hundreds of 2D IR spectra are required which correlates to several hours of spectrometer time at a minimum. In this talk I will discuss our approach to interfacing a 100 kHz repetition rate 2D IR spectrometer with microfluidic technology, thus producing a high-throughput 2D IR spectroscopy platform. The platform detailed allows roughly 800, fully averaged, 2D IR spectra of five different solvent mixtures to be collected in 30 minutes.

Keywords: Instrumentation, Lab-on-a-Chip/Microfluidics, Ultra Fast Spectroscopy, Vibrational Spectroscopy

Application Code: High-Throughput Chemical Analysis

Methodology Code: Vibrational Spectroscopy
Nonlinear spectroscopies provide valuable electronic and vibrational information, albeit the complexity of synchronizing multiple pulses at phase matching angles. Our group has been working on developing pulse shapers capable of taking ultrashort sub-10 fs pulses, and converting them into trains of pulses optimized for nonlinear spectroscopic probing. We find that chemically resolved imaging of biomedical samples becomes possible by selective electronic and vibrational excitation using trains of shaped pulses. Examples include chemically resolved imaging of pathology slides, depth resolved nonlinear spectroscopy of human skin and multiphoton excited fluorescence of red blood cells. As femtosecond laser sources decrease in price and their reliability improves, these spectroscopic imaging modalities are expected to gain acceptance in the clinic.

**Abstract Text**

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**Keywords:** Biological Samples, Biospectroscopy, Molecular Spectroscopy, Vibrational Spectroscopy

**Application Code:** Biomedical

**Methodology Code:** Microscopy
Hybrid halide perovskite solar cells now display solar power conversion efficiencies exceeding 22%. In these materials, excitonic and free-carrier regimes of primary photoexcitations are possible depending on crystalline microstructure of the active layer and excitation density. Recent literature suggests that photocarriers in these materials may be large polarons, with this notion motivated by observation that charge transport is limited by acoustic phonon scattering, and not by impurities and crystalline defects present ubiquitously in these polycrystalline microstructures. In order to explore the nature of photocarriers in these materials, we implement two-dimensional coherent photocurrent excitation (2D-PCE) spectroscopy on an optimized solar cell based on a methylammonium lead iodide perovskite. Via the time-resolved total correlation spectrum, we identify both excitonic and continuum resonances. By means of temperature dependent measurements of the rephasing zero-time spectrum, we explore the possible polaronic character of the exciton and continuum resonances and address directly whether this measurement reflects such phonon coupling.

Keywords: Semiconductor, Spectroscopy, Ultra Fast Spectroscopy
Application Code: Material Science
Methodology Code: New Method
Cold polyatomic molecules offer rich possibilities for precision measurement, quantum control, and tests of fundamental symmetries. While manipulating, cooling, and detecting atoms and certain diatomic molecules at the single quantum state level is now possible, analogous tools for controlling polyatomic molecules lag far behind. Buffer gas cooling has emerged as a surprisingly versatile tool for cooling such molecules. We will present recent demonstrations of state-specific and enantiomer-specific preparation of chiral molecules, and novel sensitive and ultra-specific chemical analysis techniques enabled by the combination of modern microwave spectroscopy techniques and the broad applicability of cryogenically buffered molecular samples.
The talk will cover devices developed in my laboratories facilitating “green” analytical procedures. In particular focus will be placed on sample preparation techniques such as SPME, Thin Film Microextraction (TFME), Needle Trap (NT) and Membrane Extraction with Sorbent Interface (MESI). Appropriate deployment of these sampling/sample preparation tools facilitate on-site deployment and provide more information about the investigated system, the features which will be highlighted. For example, by combining SPME and NTD extraction allows for the differentiation of free and particulate bound compounds in a gaseous sample matrix. Samples may contain both solid and liquid aerosols in addition to freely dissolved analytes. The NTDs trap both gaseous chemical compounds as well as particulate matter present in the sample. SPME samples only the freely dissolved analytes.

Biocompatible protection layer used in combination with sorbents is able to selectively enrich small molecules and eliminate fouling caused by macromolecules facilitating target and non-target determinations in complex biological matrices. This approach demonstrated ability to extract compounds characterized by broad range of physico-chemical properties including polarities (logP). As the results show minimal matrix effect it is suitable not only for LC applications, but also direct coupling to MS, which facilitate fast detection in different areas including clinical rapid diagnosis.

Capillary Isoelectric Focusing with Whole Column Imaging Detection (CIEF-WCID) is a very powerful approach and together with on-line sample clean-up based on dialysis hollow fiber membrane enables rapid process and QC monitoring of protein products in biotechnology industry. It is also a very powerful approach to couple CIEF to MS sine it provides on-line information about the focusing process facilitating introduction of the focused proteins into mass spectrometer.
In the days of high speed high resolution digital data acquisition, it is hard to remember that peaks were once recorded fraction by fraction, point by point. Servo motors and chart recorders thankfully ended this but even then some of us will remember cutting out peaks from a chart or a copy thereof and weighing them for accurate quantitation. Digitization first entered the world of chromatography in the form of “integrators”, which allowed facile area based quantitation. Today available chromatographic software readily allows either height or area based quantitation. As long as one is in a domain where the detector response is linearly proportional to the analyte concentration in the detection cell, the peak area is a true representation of the amount of the analyte. Height is an approximation of the area and can represent area accurately only if the peak shape remains invariant. Both height and area based approaches to quantitation reduces two-dimensional chromatographic data to one, for a set of calibration data there can be only one height or area based calibration equation for quantitation. This approach has not changed in last 50 years. High speed high resolution data acquisition, however, also permits rapid computation of the width of a peak, at any number of chosen fixed heights (here we do not mean width at fixed fraction of the peak height as in width at half-height or width at 10% of the peak height, rather the width at specified signal values above the baseline, e.g., 0.1 volt, or 1 mAU or 1 \( \text{S/cm} \), etc.), leading to any number of applicable calibration curves. We will discuss in this presentation the many ways in which width-based quantitation outperforms height or area based quantitation.
Capillary Zone Electrophoresis (CZE) is emerging as a useful tool in proteomic analysis. Interest arises from dramatic improvements in performance that result from improvements in the background electrolyte used for the separation, the incorporation of advanced sample injection methods, the development of robust and sensitive electrospray interfaces, and the coupling with Orbitrap mass spectrometers with high resolution and sensitivity. The combination of these technologies produces performance that is rapidly approaching the performance of UPLC-based methods for microgram samples and exceeds the performance of UPLC-based methods for mid- to low nanogram samples. These systems now produce over 10,000 peptide IDs in a single 100-minute analysis of the HeLa proteome.

Keywords: Capillary Electrophoresis, Mass Spectrometry, Proteomics
Application Code: Bioanalytical
Methodology Code: Capillary Electrophoresis
The advent of ambient ionization techniques brought a new era in the field analysis applications of mass spectrometry. Desorption Electrospray Ionization (DESI) and Direct Analysis in Real Time (DART) enabled the analysis of arbitrary objects without any means of sample preparation. While these methods provided solution for a number of analytical problems, their application area was constrained to surface analysis. As a response to the need for ambient bulk phase analysis new generation of ambient MS methods was developed, commonly termed as Rapid Evaporative Ionization Mass Spectrometry (REIMS). Interestingly, these methods were conceived by the direct coupling of surgical dissection methods with atmospheric ionization mass spectrometry. Surgical tissue ablation by Joule-heating, contact heating, radiative heating or ultrasonic atomization turns tissue components into an aerosol (aka. surgical smoke), which can be turned into gaseous ions in the atmospheric interface of API mass spectrometers. While the direct introduction of aerosols into unmodified instruments produces mass spectral data, further investigations revealed that the actual ion formation follows a surface-induced dissociation of scenario. Based on this mechanism, a novel atmospheric interface setup was designed and built, featuring a heated jet disruptor surface positioned at the Mach-disc region of the rough vacuum regime of the atmospheric inlet. The ion current obtained by the novel interface was found to show a dramatic dependence on the aerosol composition, which observation prompted further developments allowing the introduction of organic solvent modifiers. These modifiers can enhance ionization efficiency by 2 orders of magnitude. As the mechanistic role of the modifier is analogous to that of matrix compound in case of MALDI, it’s generally referred to as matrix.

Keywords: Biological Samples, Biomedical, Clinical Chemistry, Mass Spectrometry
Application Code: Biomedical
Methodology Code: Mass Spectrometry
Capillary liquid chromatography has advantages when samples are small. We have endeavored to improve sample loading and separations performance by actively controlling the column temperature at discrete times and places along the column. Most commonly, it is at the injection end of the column where we can improve solute focusing. We have also used active temperature control to alter separation performance for investigations into neuroscience, specifically in the determination of ectopeptidase activity in brain and for the determination of neurotransmitters by online capillary LC/microdialysis with one-minute resolution.
During food processing, contaminants can be produced through heat and reactions between food components. In some cases, the production of these compounds is unavoidable and in others additional steps can be taken by the manufacturer to reduce the amounts in the final food products. Many of these compounds are potentially harmful and some have regulatory limits set by the FDA. Benzene, chloropropanols, acrylamide, and furans have all been investigated in a wide range of foods and beverages in recent years. Benzene can be produced in beverages containing both ascorbic acid and the preservative sodium benzoate. Chloropropanols are produced with the reaction of residual lipids and hydrochloric acid in the production of “chemical” soy sauce and other thermally processed foods such as cereals and meat. Furans have been investigated in canned and jarred foods. Acrylamide can be found in carbohydrate rich food and is produced during the baking or roasting process. Research on these compounds including analytical methods and market sampling results will be presented. Additionally, due to the thermally labile nature of these compounds, challenges associated with these methods will also be discussed.
Fatty acid esters of 3-chloro-1,2-propanediol (3-MCPD), 2-chloro-1,3-propanediol (2-MCPD), and glycidol are process-induced chemical contaminants found in refined edible vegetable oils. Formed during the deodorization step of the refining process, these compounds are considered potentially carcinogenic and/or genotoxic, making their presence in edible oils and processed foods containing these oils a potential health risk. Because refined oils serve as the primary fat source in infant formula, and are also major components in other complex food matrices consumed by infants and toddlers, there is a need for methodology to detect MCPD and glycidyl esters in these foods in order to determine levels of exposure.

A novel approach for extracting and detecting MCPD and glycidyl esters in infant formula will be described in this presentation. Quantitation of the esters was performed using a LC-MS/MS method that was previously validated for the quantitation of these species in edible oils. Results of the extraction and quantitation indicate ester recoveries ranging between 85 and 115% can be achieved. Using this validated methodology, occurrence data for 3-MCPD and glycidol was produced for a number of commercially available infant formulas in the United States and Europe. In addition, this presentation will discuss the extension of this method to several other complex food matrices commonly consumed by infants and toddlers.

Keywords: Extraction, Liquid Chromatography/Mass Spectroscopy, Sample Preparation, Tandem Mass Spec
Application Code: Food Contaminants
Methodology Code: Sampling and Sample Preparation
In the past, many studies have been undertaken to elucidate the key odorants of food and to identify formation pathways of the so-called “food-borne toxicants”. But, up to now, analytical approaches including the quantitation of desirable aroma-active compounds in combination with undesirable toxicologically relevant substances by sensitive methods are scarcely available.

The lecture will present recent studies, which were combining the analysis of important aroma compounds and of selected food-borne toxicants (e.g., acrylamide, acrolein, crotonaldehyde, styrene, etc.) formed during food-processing, e.g., brewing of beer or deep-frying of potato chips and donuts in different edible oils. Odorants were identified by gas chromatography-olfactometry as well as GC-MS and quantitated by stable isotope dilution analysis (SIDA). For the toxicants, new quantitation methods using stable isotopically labeled standards (e.g., [13C3]-acrolein or synthesized [13C4]-crotonaldehyde) were developed and formation pathways were proven by labeling experiments.

In summary, it will be shown that lowering the amounts of undesirable compounds in combination with the maintenance of an overall aroma well accepted by the consumers is a challenging task.

Keywords: Flavor/Essential Oil, Food Contaminants, Gas Chromatography/Mass Spectrometry, Liquid Chromatography
Application Code: Food Contaminants
Methodology Code: Gas Chromatography/Mass Spectrometry
Furosine [N-(2-furoylmethyl)-L-lysine] is generated in the early stages of the Maillard reaction through the condensation of reducing sugars with the ε-amino group of protein-bound lysine. Formation of furosine produces a loss in the nutritional availability of lysine, making furosine a suitable marker for monitoring protein damage in thermally processed foods.

In tomato processing furosine can be used as a heat damage index to monitor and control thermal parameters during production. Due to the polarity of the molecule, furosine typically requires ion pairing reagents to improve retention for separation by LC analysis. Use of ion-pairing chromatography can cause contamination of mass spectrometers leading to long term interferences with other analyses. In this presentation, a reversed-phase LC-MS analysis will be discussed which uses ion pairing in sample preparation to minimize contamination of the mass spectrometer.
How Did That Get in My Food? Determination of Process Induced Food Contaminants

4-Methyl Imidazole - From Formation and Analysis to Toxicology and Regulatory Status

The Maillard browning reaction between amino acids and reducing sugars gives roasted, grilled and baked foods their enticing aromas and desirable flavors and color. The reaction is also responsible for the generation of compounds deemed less desirable, like 4-methyl imidazole, among others. 4-Methyl imidazole (4-MEI) is a heterocyclic compound (C4H6N2) formed during Maillard browning and in some caramel color manufacturing processes. In Maillard browning reactions, 4-MEI is believed to be formed from reactions of alpha-dicarbonyl compounds generated from carbohydrates with ammonia from Strecker degradation of amino acids. Caramel colors (used, for example, in soft drinks, beer, syrups, sauces, gravies and seasonings) are also a source of 4-MEI in foods. Caramel colors are classified based on the reactants used in the manufacturing process. While there are four classes of caramel colors, Classes III and IV are processed in the presence of ammonia, which can generate 4-MEI. A U.S. National Toxicology Program study in 2007 concluded that 4-MEI caused cancer in mice after a two year feeding study where mice received daily doses of 80 - 170 mg of 4-MEI per kilogram of body weight in their feed. In 2011, it was listed by the California Environmental Protection Agency as a Prop 65 carcinogen in California. Caramel colors, a major source of 4-MEI in foods, are considered safe by the U.S. Food and Drug Administration, the European Food Safety Authority and Health Canada. This presentation will provide an overview of 4-MEI, its formation and analysis in food, the toxicological implications and current regulatory status.

Keywords: Food Contaminants, Food Science, Liquid Chromatography/Mass Spectroscopy
Application Code: Food Contaminants
Methodology Code: Liquid Chromatography/Mass Spectrometry
Over the last 40 years in vivo voltammetry has evolved from a pipe dream to a reliable analytical method used by a variety of scientists ranging from analytical chemists to psychiatrists. The most widely used technique in this field is cyclic voltammetry at carbon-fiber microelectrodes. Cyclic voltammetry provides sufficient sensitivity and chemical selectivity to detect fluctuating neurotransmitter concentrations in behaving animals. Furthermore, this technique can readily be combined with others such as single unit recordings and iontophoresis. The combined approaches provide considerable information on the mode of neural communication employed by the neurotransmitter.
Electrochemical cytometry and mass spectrometry imaging with NanoSIMS have been used to peer into the contents and even the substructure of single neurotransmitter vesicles in pheochromocytoma cells. We have discovered that a chemotherapeutic drug that decreases cognition, cisplatin, changes exocytosis, but not vesicle content. We have discovered that the learning supplement, zinc ion, decreases vesicle content, but not exocytosis by changing the fraction that is released. We have discovered that dietary supplement, curcumin, changes the rate of exocytosis, but not the amount stored in vesicles. We have discovered that the anaesthetic, lidocaine, does not change the amount of dopamine stored in vesicles, but decreases the amount released during exocytosis. We have used intracellular cytometry to measure the content of small synaptic vesicles (50-60 nm) in the nerve terminals of the living fruit fly larva and found the amount to be orders of magnitude higher than that measured during exocytosis release.
Carbon-fiber microelectrodes have been the fundamental tool for the electrochemical detection of neurotransmitters since their introduction in the early 1980s. They are biologically compatible and, compared to larger electrodes, microelectrodes offer highly increased diffusional flux to the electrode surface and faster response times to enable high speed measurements. The demonstrated benefit of enhanced mass transport to very small electrodes has led us to develop several new carbon-based microelectrodes for the improved detection of dynamic neurochemical fluctuations. This talk will describe the characterization of a recessed microdisk electrode for enhanced detection of individual exocytosis events at single cells, a nanoscale microelectrode with a conical geometry for fast intracellular voltammetric measurements, and innovative yarn-like sensing substrates engineered entirely of multi-walled carbon nanotubes for enhanced sensitivity. Each of these ultramicroelectrode designs exploits mass transport to either provide maximal diffusional flux to, or to limit diffusional flux from, the electrode surface. These new tools provide significant advantages when coupled with fast-scan cyclic voltammetry, and research using them to provide new insight into in vivo molecular dynamics will be discussed.

Keywords: Bioanalytical, Electrochemistry, Microelectrode, Neurochemistry
Application Code: Neurochemistry
Methodology Code: Electrochemistry
In Vivo Neurochemistry: Faster, Smaller, More Sensitive Methods for Real-Time Neuroanalysis

New Insights into DA from New Kinetic Models

Fast-scan cyclic voltammetry allows one to measure the release and clearance of several neurotransmitters, including dopamine, by neuronal terminals in various brain regions. One common application is the use of voltammetry to monitor realtime release and clearance evoked by electrical stimulation of dopamine axons. Interpretation of such evoked responses often depends upon the availability of a kinetic model to resolve the underlying release, clearance, and mass transport processes that combine to determine the amplitude, duration, and temporal features of the data. Several such models have been reported in the literature based upon different kinetic postulates and these provide different levels of agreement with measured data and utilize different numbers of adjustable parameters. Here, we report recent progress with a newer model we have developed that we call the Restricted Diffusion Model.

Keywords: Bioanalytical, Electrochemistry, Neurochemistry, Voltammetry
Application Code: Neurochemistry
Methodology Code: Electrochemistry
**In Vivo Neurochemistry: Faster, Smaller, More Sensitive Methods for Real-Time Neuroanalysis**

**Tunable CNT Fiber and Yarn Microelectrodes**

CNT fiber microelectrodes are highly desirable for tissue applications because of their small size and superior electrical and mechanical properties. Instead of modifying an existing electrode, CNT fibers can be directly fabricated as electrodes in a manner similar to conventionally used carbon-fiber microelectrodes (CFMEs) with high reproducibility. In this study, we fabricated microelectrodes using three CNT fibers assembled by different protocols, including CNT wet spinning with polyethylenimine (PEI/CNT) or chlorosulfonic acid (CA/CNT), and solid based CNT drawing (CNT yarn). Using fast-scan cyclic voltammetry, CNT yarn microelectrodes exhibit the highest current density and smallest $E_p$ value for dopamine detection. CNT yarns are advantageous because of their relatively abundant defects sites and oxygen containing functional groups as well as better alignment of CNTs. CNT yarn and PEI/CNT fiber microelectrodes are more reversible for dopamine and have a current that is independent of sampling rate, indicating that they can be used with high temporal resolution. PEI/CNT fiber microelectrodes are more selective to DOPAC and CNT yarn microelectrodes show significantly higher selectivity to serotonin than other fibers. CA/CNT fiber microelectrodes have the highest selectivity of dopamine to ascorbic acid and uric acid, because of the negative charge carried by CA. Overall, CNT yarns offer fast time response and better reproducibility while PEI/CNT and CA/CNT fibers could be used when selectivity to DOPAC or selectivity to cations over anions is desired, respectively. In addition, we have explored surface treatments of microelectrodes, including laser treatment, oxygen plasma treatment, and antistatic gun treatment. These treatments increase the sensitivity and change the selectivity of the sensors, allowing the properties to be tuned. Thus, different CNT fibers can be used to tune the electrochemical properties for real-time, in vivo neurotransmitter detection.

**Keywords:** Electrochemistry, Neurochemistry, Optogenetics, Voltammetry

**Application Code:** Neurochemistry

**Methodology Code:** Electrochemistry
A current trend of bioanalysis is for deeper understanding of life phenomena and practical application to medical fields. To analyze the biological events in single cells, technologies related to fluorescence and bioluminescence imaging have been advancing rapidly in the past decade. Remarkable advancement has been achieved in the visualization 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. In particular, novel design of fluorescent and bioluminescent proteins has achieved great breakthrough in the field of molecular imaging and drug development.

I herein focus on new luminescent sensors using engineered green fluorescent proteins (GFPs) and luciferases for the analysis of living cells and mice; the principle is based on complementation and reconstitution of the split-reporter fragments when they are brought sufficiently close together. To show the applicability, I demonstrate imaging technologies of apoptosis, caspase activities and GPCR-arrestin interactions. The sensors for GPCR-arrestin interactions were used further for identifying the specific inhibitors screened from large-scale chemical library. Such small organic molecules will become potent candidates for new chemical tools and lead compounds for drug development. I also show novel techniques of light control of a kinase activity in living cells, which will be useful for planning temporal patterns of chemical doses. These techniques will engender deeper understanding of biological systems and will break new ground in pharmacological analysis.

Reference

Keywords: Bioanalytical, Fluorescence, Imaging, Luminescence
Application Code: Bioanalytical
Methodology Code: Fluorescence/Luminescence
Biopolymers are highly complex and large molecules. Most proteins are expressed in several isoforms. Isoforms differ in modifications of individual amino acid side chains, or the N- or C-terminus. Typical modifications are deamidation, phosphorylation, acetylation, methylation, oxidation, or glycosylation. Isoforms may differ in biological activity and stability. Therefore, a thorough characterization and quantification of the isoforms is needed to ensure consistent product quality. Fast separation of monoclonal antibody isoforms is important for profiling and mass spectrometric determination. Here we report the workflow solutions for the for speedy, productive and accurate separation of complex heterogenic species of monoclonal antibodies and their conjugates using different modes of analytical chromatography by HPLC and UHPLC.

Keywords: Bioanalytical, HPLC, Liquid Chromatography
Application Code: Bioanalytical
Methodology Code: Liquid Chromatography
Demand is increasing for protein quantitation in biological fluids for disease detection, protein therapeutics monitoring, and drug development response control. Current methods use highly sensitive and specific triple quadrupole mass spectrometry (QqQ-MS) to quantify protein digest peptides. These bottom-up methods require protein digestion, which is often incomplete and can introduce errors in protein quantitation. Therefore, absolute protein quantitation is impossible without including expensive isotopically labeled protein standards. In our lab, a method that bypasses the protein digestion step to directly quantify intact proteins on QqQ-MS was developed. Myoglobin, cytochrome c, lactalbumin, lysozyme, and ubiquitin were used as protein standards to prove the principle. The method was developed on a Shimadzu LCMS-8050 QqQ-MS using multiple reaction monitoring (MRM). Inconsistent and irreproducible intact protein fragments were a challenge initially; however, a few reproducible and intense product ions were found using a lower collision energy and high collision gas pressure. MRM transitions for all protein standards were developed and calibration curves were obtained with respectable linearity (R²>0.99). In order to address complex matrices in biological fluids for future applications, a generic reversed-phase chromatography method was developed on widepore Restek Viva (2.1 x 100 mm; 5 μm; 300 Å) columns. Prostate specific antigen (PSA) was also included in the study to prove the feasibility of the method for both of the liquid chromatography and mass spectrometry aspect. Specificity of the MRM detection were evaluated for urine and plasma matrices. The method is envisioned to be a model for future development of targeted methods for analysis of important disease indicators such as proteins in biological fluids, especially for biopharmaceuticals, clinical diagnostic and treatment advancements.

Keywords: Liquid Chromatography/Mass Spectroscopy, Protein, Quadrupole MS, Quantitative
Application Code: Bioanalytical
Methodology Code: Liquid Chromatography/Mass Spectrometry
New technological advances in the detection of inorganic materials have changed its relevance in the biopharmaceutical field. The utilization of ICP-QQQ for the ultra-trace analysis of protein-metal conjugates is allowing for the determination of biomolecules at levels that are biologically relevant opening the door to enhance investigations in the areas of biological research. The ground-breaking Agilent 8900 ICP Triple Quad (ICP-QQQ) has transformed the ICP-MS landscape with its MS/MS technology. The growing acceptance of the ICP-QQQ has been highlighted in many Bio Pharmaceutical publications where all its benefits have been enumerated. In this session, we will introduce latest Bio Pharmaceutical Application by ICP-QQQ.

**Keywords:** Atomic Spectroscopy, Biopharmaceutical, ICP-MS, Pharmaceutical

**Application Code:** Pharmaceutical

**Methodology Code:** Atomic Spectroscopy/Elemental Analysis
qNMR (quantitative NMR) using internal standard method, named as AQARI (Accurate QuAntitative NMR with Internal standard material), has started to be used as an official analytical method, including by the Japanese Pharmacopoeia and the Japanese Standard of Food Additives, because of its high degree of reliability and SI traceability. We investigated the potency of AQARI to apply the quality assurance of analytical standards which in used for Gas Chromatography and Liquid Chromatography. In this application, we used Wako’s Internal Standards for qNMR, CIL’s Deuterated Solvents and JEOL’s NMR instrument of JNM-ECS400, JNM-ECA600. As a result, we were able to implement the purity determination of analytical standards for more than 500 products, including seven Japanese Pharmacopoeia crude drug products, magnolol, geniposide, paeonol, magnoflorine iodide, saikosaponin b2, (E)-cinnamic acid and rosmarinic Acid. In the future, AQARI is planned to be applied to the JIS (Japan Industrial Standards) as a high accurate analytical method with SI traceability.
The realization of label-free molecule specific imaging in real time is crucial for precise surgical guidance and intraoperative histopathologic examination of tissue. Thus, new approaches for an intraoperative tissue characterization to supplement routine pathological diagnostics is needed. In this context Raman imaging is particularly noteworthy since it can provide a pathologist with clinically-relevant information under both ex vivo and in vivo conditions. While the advantage of Raman spectroscopy is its unprecedented high specificity it suffers from its poor sensitivity. This disadvantage can be overcome by utilizing non-linear Raman phenomena like CARS = coherent anti-Stokes Raman scattering or SRS = stimulated Raman scattering. In this contribution we shall discuss the application and potential of non-linear Raman-imaging for spectral histopathology. However, these methods as standalone techniques are typically highlighting only a particular detail of pathological alterations. In order to improve the diagnostic result the combination of Raman approaches with other optical and spectroscopic imaging techniques in a multimodal approach is very beneficial. Here we will show, that multimodal nonlinear microscopy combining CARS, two-photon excited autofluorescence (TPEF) and second harmonic generation (SHG) enables the detection of characteristic structures and the accompanying molecular changes of widespread diseases. To facilitate handling and interpretation of the image data characteristic properties can be automatically extracted by advanced image processing algorithms. Overall, the presented examples show the great potential of this multimodal imaging approach together with advanced image analysis routines to overcome current limitations of frozen section analysis with respect to achieving a reliable intraoperative tumor margin detection.
Multimodal information is beneficial for characterizing and understanding the composition and function of chemical interfaces. Here we show the use of a hybrid AFM/IR/MS imaging platform for the acquisition and precise correlation of nanoscale surface topography and chemical images from both IR and MS. The MS-based chemical imaging component of the system utilized nanothermal analysis probes for thermal desorption or thermolytic surface sampling followed by APCI or ESI of the gas phase species produced with subsequent mass analysis. The basic instrumental setup, operation, and image correlation procedures are discussed and the multimodal imaging capability, spatial resolution and utility are demonstrated using various natural and synthetic polymers and other materials. The experiments were accomplished using a nanoIR2™ AFM instrument (Anasys Instruments, Santa Barbara, CA, USA) and a LTQ XL mass spectrometer (Thermo Fisher Scientific, Waltham, MA). Material liberated from the polymer surface via thermolysis induced by the heated nano-TA AFM probe (model PR-EX-AN2-300, Anasys Instruments) was ionized and transferred to the mass spectrometer via an in-line vapor extractor/corona discharge APCI source. IR imaging experiments utilized detection of photothermal expansion of the sample at the tip of the nanoIR2™ AFM probe (model PR-EX-NIR2, Anasys Instruments) recorded at IR wave numbers specific to the different surface constituents. Topography and chemical images were correlated using an in-house developed software package (MultiDimChemImaging).

**Keywords:** Atomic Force Microscopy (AFM), Imaging, Infrared and Raman, Instrumentation

**Application Code:** Material Science

**Methodology Code:** Mass Spectrometry
Biofilms, such as those formed by the opportunistic human pathogen \textit{Pseudomonas aeruginosa}, are complex, matrix enclosed, surface-associated communities of cells that exhibit properties - such as enhanced resistance to antibiotics - distinct from their free-floating counterparts. \textit{P. aeruginosa} biofilms are associated with persistent and chronic infections in diseases such as cystic fibrosis and HIV-AIDS. Communities of \textit{P. aeruginosa} organize themselves by synthesizing and secreting signaling molecules, implicated in quorum sensing (QS), and in regulating biofilm formation and virulence. Correlating chemical information from powerful molecular imaging platforms, such as confocal Raman microscopy (CRM) and SIMS-based mass spectrometric imaging in conjunction with multivariate statistical tools such as principal component analysis is being applied to study the spatial and temporal distributions of signaling molecules, secondary metabolites and virulence factors in biofilm communities of \textit{P. aeruginosa}. These studies reveal that laboratory strains of \textit{Pseudomonas} differ significantly both from genetic mutants and from clinical isolate strains in the mechanisms used for chemical communication as well as the organization of the resulting biofilms in monoculture. They also reveal significant modes of action in co-cultures involving different strains and different bacterial species.

**Keywords:** Microscopy, Microspectroscopy, Raman Spectroscopy

**Application Code:** Bioanalytical

**Methodology Code:** Molecular Spectroscopy
Multimodal Chemical Imaging Approaches

New Approaches for Multimodal Ambient Imaging of Biological Samples

Multimodal imaging plays a critical role in unraveling the complexity of biological systems. We have developed a unique platform for imaging biological systems by coupling nanospray desorption electrospray ionization mass spectrometry imaging (nano-DESI MSI) with other imaging modalities including shear force microscopy (SFM), scanning ion conductance microscopy (SICM), and scanning electrochemical cell microscopy (SECM). Nano-DESI MSI enables minimally invasive quantitative MSI of hundreds of endogenous molecules in biological samples using localized liquid extraction and is ideally suited for studying fully hydrated biological systems in their native states (e.g. living microbial communities, cells and tissues). Coupling nano-DESI MSI with SFM enables simultaneous chemical and topographic imaging of complex biological samples. Meanwhile SICM and SECM enable contact-free mapping of the cell topography, studying protein dynamics at the nanoscale, and quantitative imaging of selected redox-active species including reactive oxygen species (ROS) and redox-active metabolites in complex biological systems. Coupling of nano-DESI MSI with SFM, SICM, and SECM is achieved using finely pulled precisely positioned nanopipettes. The design and performance of the new imaging platform will be presented and multimodal imaging of tissue sections and individual cells immobilized on glass slides will be demonstrated.

Keywords: Biological Samples, Electrospray, Imaging, Mass Spectrometry
Application Code: Bioanalytical
Methodology Code: Mass Spectrometry
With recent advances in analytical instrumentation and data capture/processing, the ability to generate comprehensive chemical maps (or “images”) of solid samples with high spatial resolution is being realized by a number of groups. In particular, unambiguous identification of non-targeted analytes within these complex samples necessarily requires multiple analytical approaches performed on the same sample; these types of methods have been termed ‘multimodal chemical imaging’ approaches. The initial multimodal imaging tools relied on non-destructive, optical spectroscopies, such as Raman and infrared spectroscopy. While these sorts of tandem imaging methods provide a wealth of information on the exact same sample with adequate spatial resolution, they often suffer from weak sensitivity, poor selectivity, or compromised spatial resolution as dictated by the spectroscopic method employed. Greater success in comprehensive chemical imaging has been achieved with instrument based around mass spectrometry (MS), due to the excellent sensitivity and selectivity; however, MS imaging is inherently destructive in nature.

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) at high spatial resolution (e.g., better than 100 microns). This capability is achieved through focused laser sampling of the solid material. The aerosol generated from the ablation event is swept to a flowing atmospheric-pressure afterglow molecular ionization source, which desorbs and ionizes molecules from the ablated particles for detection by mass spectrometry. In addition, light from the laser-induced plasma is collected and detected to provide optical emission information on the elements in the sampled spot. The design of this instrument will be discussed in detail along with preliminary analytical figures-of-merit.

Keywords: Laser, Mass Spectrometry, Organic Mass Spectrometry, Plasma Emission (ICP/MIP/DCP/etc.)
Application Code: General Interest
Methodology Code: Mass Spectrometry
Identifying small-molecule binders to protein targets remains a daunting task due to the huge diversity in compound structure, activity, and mechanisms of action. Affinity-based target ID techniques are limited by the necessity to modify each drug individually (without losing bioactivity), while non-affinity based approaches are dependent on the drug’s ability to induce specific biochemical/cellular readouts. To overcome these limitations, we have developed a high-throughput LC-MS platform coupled to a universally applicable target identification approach that analyzes direct small-molecule binding to its protein target(s). Once protein targets to specific ligands are revealed, native MS and ion mobility can be used to interrogate their binding stoichiometry and affinity. We use high resolution Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) to perform top-down MS to probe ligand-binding sites. We are using electron capture dissociation (ECD)/FT-ICR MS to investigate the molecular action of compounds that prevent amyloid fibril formation in neurodegenerative diseases such as Alzheimer’s and Parkinson’s disease. Other means for activation/dissociation, including infrared multiphoton dissociation (IRMPD), ultraviolet photodissociation (UVPD), and electron ionization dissociation (EID) generate complementary structural information.
Enhancing Metabolomic Analyses with Automated Solid Phase Extractions and Ion Mobility-Mass Spectrometry

Metabolomic analyses of biofluid and environmental samples 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 metabolite. However, ionization suppression is readily observed in IMS-MS direct injection studies of complex samples due to the numerous components in plasma and the high salt concentrations in urine. Thus, a rapid separation is often necessary prior to 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. Results from both environmental and biological samples will be illustrated in this presentation.

Keywords: Bioanalytical, High Throughput Chemical Analysis, Mass Spectrometry, Metabolomics, Metabonomic

Application Code: Bioanalytical

Methodology Code: Mass Spectrometry
Opportunities in ‘small molecule’ applications include rapidly generating theoretical collision cross-sections from molecular modelling and utilising them for identification and confirmation by comparison with experimental values from ion mobility mass spectrometry. This is particularly valuable when there are isomers and MS/MS is not able to distinguish between them or for an additional identification point. Using molecular modelling it is also possible to postulate the most likely bonds that undergo cleavage during MS/MS. An overview of studies on a range of ‘small molecules’ particularly relevant to food and environmental and pharma applications is presented.
Inhibition of Lactate Export Paradoxically Transforms Mitochondria from Synthesis Organelles to Oxidative Machines: Insights from a MIMOSA-Based Fluxomics Screen

Fluxomics is an approach to study metabolism that usually involves feeding cells stable-labelled precursors (commonly 13C labelled metabolic intermediates) and following the resulting isotopomers through biochemical pathways using LC/MS/MS. The Kibbey lab (Yale School of Medicine) has developed the Mass Isotopomeric Multi-Ordinate Spectroscopic Analysis (MIMOSA) method which uses differential ion mobility DMS-LC/MS/MS technology to detect and quantitate these labelled metabolites. DMS technology acts orthogonal to LC to achieve separation of isobaric and structurally similar molecules. It captures both steady-state and dynamic metabolic fluxes by resolving positional isotopomers of glycolysis and the TCA cycle. We have recently engaged in a formal collaboration with the Kibbey lab (Yale) to bring this cutting edge technology to Pfizer. Here we discuss the proof-of-concept/pilot study that was conducted with the Oncology RU on the lactate transporter MCT1. Monocarboxylic acid transporters (MCT) have been identified as a possible target for cancer therapy. Studies have shown that blocking lactate transport by inhibiting MCT1 causes growth arrest in cancer cell lines. However, the impact of MCT1 inhibition on central carbon metabolism (which may produce untoward effects) is unknown. Our goal with this fluxomics experiment was to discover the steady-state metabolic changes that occur in response to the inhibition of lactate secretion in a MCT1-expressing cancer cell line. Cell cultures were treated with AR-C15585, a commercial MCT1 inhibitor (MCT1i) at 20 nM and [U-13C6]glucose for 4 h, then analyzed using the MIMOSA approach. The treatment with MCT1i resulted in 1). the expected accumulation of lactate, 2). a shift in intracellular redox, 3) a shift from anaplerotic (synthetic) metabolism to oxidative metabolism (either by decreasing PC, by increasing PDH, or both), and a decrease in cellular nitrogen availability in the form of ASP & GLU.

Keywords: Bioanalytical, Chromatography, Mass Spectrometry, Metabolomics
Application Code: Bioanalytical
Methodology Code: Liquid Chromatography/Mass Spectrometry
Mass spectrometry (MS) is a routine biophysical analytical technique used within pharmaceutical research. The same cannot yet be said for native-MS and ion mobility (IM). Within this presentation, it will be demonstrated how MS, and especially native-MS and IM, and complimentary analytical techniques, can be applied to many analytical problems within pharmaceutical research today.

Diastereoisomers such as beta and dexamethasone and the isomeric pairs of cis and trans proline can be separated and differentiated in the gas-phase by IM (both drift-tube and travelling wave). We also show that with the use of molecular dynamics (MD), quantum mechanical (QM) calculations and the use of different IM drift-gases, the ion behavior can be completely rationalized.

Examples will also be described for how MS can be applied to drug discovery using a high-throughput (HT) platform which affords the rapid screening of multiple reactive acrylamide-based small molecules against a cysteine-containing therapeutic target of interest. The HT-platform will also be used to rapidly screen monoclonal antibodies (reduced and intact) and accurately determine the drug antibody ratios (DARs) of antibody drug complexes (ADCs).

In conclusion, we will also demonstrate how native-MS, native top-down MS, MD (both all-atom and coarse-grain) and native-IM have been used to characterize the gas-phase collapse of an IgG1 monoclonal antibody.

Keywords: Biopharmaceutical, Light Scattering, Mass Spectrometry
Application Code: Bioanalytical
Methodology Code: Mass Spectrometry
Photoelectrochemical solar energy conversion to electricity or fuels is often limited by the activity of photogenerated holes and electrons on photoelectrode surfaces, requiring modification with catalysts to improve efficiency. It is unclear, however, on what surface sites to optimally deposit catalysts, and to what extent the same surface sites, responsible for fuel-forming reactions, also cause recombination. Here we use charge-carrier-selective single-molecule super-resolution photoelectrocatalysis imaging to quantify the correlation between photogenerated hole and electron activities on semiconductor surfaces at nanometer resolution under photoelectrochemical water oxidation conditions. We further use sub-particle-level photocurrent-vs-potential measurements to define the relationship between water oxidation photocurrent and charge-carrier surface activities. Guided by hole and electron activity maps, site-selective catalyst deposition on individual semiconductor particles reveals the optimal sites for oxygen evolution catalysts, leading to a strategy for rationally engineering photoanodes with catalysts.

Keywords: Electrochemistry, Energy, Fluorescence, Microscopy
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Microscopy
Studying electrochemical reactions on single nanoscale electrodes is a distinct challenge using traditional electrochemistry techniques because it is difficult to electrochemically address a single nanoparticle electrode and the currents produced are often below the signal-to-noise of the measurement system. Optical readouts of electrochemistry are an attractive alternative provided one can find a reporter molecule which changes its optical signature upon a change in its oxidation state. This talk will describe strategies for optically imaging electrochemical readouts using surface-enhanced Raman scattering (SERS), fluorescence, and/or electrogenerated chemiluminescence in order to visualize electrochemical reactions on nanoparticle electrodes.

Keywords: Spectroelectrochemistry, Surface Enhanced Raman Spectroscopy
Application Code: Nanotechnology
Methodology Code: Electrochemistry
In this talk, I will describe our research on electrochemical oxidation and dissolution of single silver nanoparticles. We have discovered an interesting electrochemical behavior during oxidation and electrodissolution of single nanoparticles on an ultramicroelectrode (UME). Specifically, we observed the presence of multiple ultrafast amperometric peaks associated with single nanoparticle oxidation. These current peaks were driven by fast and repeated nanoparticle collision and partial dissolution of single nanoparticles in the vicinity of the electrode surface. Interestingly, the total charge integrated from all the current peaks was found to be less than that predicted from the size of silver nanoparticles indicating partial oxidation. Our results have revealed some of the complex nature of single nanoparticle dissolution during collision on an UME surface.
Investigations of electrochemistry (e.g. voltammetry or chronoamperometry) at the single nanoparticle (NP) level present huge technical and instrumentation challenges, arising from the need to measure small current signals (usually several pA or smaller), from individual nanoscale entities, with high time resolution in order to access dynamic behavior. In this contribution we will present developments on 2 fronts that are addressing these issues head on:

(i) The use of scanning electrochemical microscopy (SECCM) as a key tool for electrochemical nanoscience, which makes use of a micropipette or nanopipette, containing electrolyte and counter/reference electrodes to create an electrochemical cell by meniscus contact with an electrode surface of interest. This versatile setup not only allows the study of single NPs on unusual supports (such as transmission electron microscopy grids, for complementary high resolution imaging), but provides high signal/noise and high bandwidth for ultrasensitive measurements. SECCM opens up the study of phenomena inaccessible previously, for example, during the impact (landing) of individual electroactive NPs on an otherwise inert electrode. We have been able to study the formation of metal NP surface oxides and their effect on electrocatalytic reactions for the first time, and the mechanism of NP electrodissolution. The high time resolution allows these processes to be analyzed in unprecedented detail, and uncover major new (and surprising) phenomena.

(ii) The use of high-speed nanoscale electrochemical mapping to probe the electrocatalytic activity of individual NPs with sub-NP resolution, such that the activity can be related to facets within a NP, not just the NP as a whole. This methodology and the information it provides has the potential to aid in the design and synthesis of optimal NP electrocatalysts, through the identification of active sites and facets.

Keywords: Electrochemistry, Electrode Surfaces, Imaging, Nanotechnology

Application Code: Nanotechnology
Methodology Code: Electrochemistry
Session Title: Single Nanoparticle Electrochemistry

Abstract Title: Resistive Pulse Delivery of Single Nanoparticles to Electrochemical Interfaces

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Abstract Text:
An experimental system for controlling and interrogating collision dynamics of individual nanoparticles at electrode/electrolyte interfaces will be presented. A glass nanopipette precisely positioned over an electrode surface (typically separated by 100 nm to 10 micrometers of solution) is used to delivery individual nanoparticles, via pressure-driven solution flow or electrophoresis, to the underlying electrode, where the nanoparticles undergo collision and are detected electrochemically. The nanopipette also serves the dual role of sizing the particle via resistive pulse sensing, as well as controlling the nanoparticle velocity and, thus, interfacial collision force. The ability to delivery one particle at a time to the interface allows correlation between particle size and catalytic activity.

Keywords: Electrochemistry
Application Code: High-Throughput Chemical Analysis
Methodology Code: Electrochemistry
The terahertz (THz) frequency region from 10^{-11} to 10^{-13} Hz has several advantages in spectroscopy, such as good transparency for optically opaque materials and sub-millimeter spatial resolution for imaging, and it can provide significant information about intermolecular interactions of hydrogen bonds between molecules as well. Although improvements are required in many respects, quantitative analyses based on THz spectra point to promising directions in the application of THz imaging to pharmaceuticals and medical diagnostics [1]. When several kinds of molecules are mainly connected by hydrogen bonds, the intermolecular vibrations appear as peaks in THz spectra, and the peaks reveal the “intermolecular fingerprint.” For the analysis of the “intermolecular fingerprint”, we developed two different types of THz spectroscopy systems. One is a THz time-domain spectroscopy (THz-TDS) system that uses a 10-fs pulse laser for wide spectral range measurement. The other is a THz frequency domain spectroscopy (THz-FDS) system that uses a uni-traveling-carrier photodiode (UTC-PD) with two 1.5-µm continuous-wave lasers for fast and stable measurements. Using the systems, we obtained images of pharmaceutical cocrystals for the first time [2,3]. A pharmaceutical cocrystal is a crystalline single-phase material composed of two or more different molecular compounds generally in a stoichiometric ratio, which are neither solvates nor simple salts. Furthermore, we achieved quantitative imaging analysis of cocrystals contained in conventional cellulose pellets using THz spectroscopy [4]. The THz-FDS system is compact and can build an image at each peak frequency very quickly. Moreover, when the system included a homodyne integrated photonic circuit, double-beam paths for a sample, and a reference, it provided effective signal stabilization for measuring glucose hydration [5].


Keywords: Biospectroscopy, Chemical, Pharmaceutical, Vibrational Spectroscopy
Application Code: Pharmaceutical
Methodology Code: Vibrational Spectroscopy
Terahertz Time-Domain Spectroscopy (THz-TDS) has made non-destructive characterization of material possible in the broadband frequency range between 0.1 and 10 THz. Many industrial applications of the THz-TDS technique have been proposed for process control in the pharmaceutical, aerospace, and chemical fabrication industries, mostly relying on the distinct vibrational modes of the materials or the time-of-flight analysis in imaging applications. These NDE techniques are usually limited to spectroscopy of chemicals in polycrystalline form, or to the thin-film detection with axial-resolutions on the order of μm. However, polarization-sensitive characterization of materials in the THz regime has not been explored as widely in the NDE applications mostly due to the need for use of wire-grid polarizers, which have a limited bandwidth performance.

We present a new THz polarization measurement instrument, in which no additional optical devices are needed compared to traditional THz-TDS systems. In THz-TDS, nonlinear crystals, such as ZnTe and GaP, are routinely utilized for detection of terahertz signals through the electro-optic sampling technique. Here, by continuous rotation of the nonlinear crystal in a perpendicular direction to the propagation of the THz and probe beams, we can resolve the absolute terahertz polarization direction without the limitations of previous techniques. Together with the ability to control the polarization direction of the ultra-broadband terahertz radiation from gas plasma sources through external biasing of the ionization region, we present a full-polarimetry technique, dubbed Terahertz Time-Domain Polarimetry (THz-TDP). THz-TDP allows for measurement of the full Stoke’s vectors of the terahertz field in the broadband range between 0.5 and 10 THz. We will show the application of this technique for characterization of ultra-thin films (μm) in non-destructive testing of multi-layer coating systems for industrial applications.
THz imaging has shown strong potential for differentiation of cancerous and healthy tissue on the surgical edge of freshly excised human breast cancer. This work demonstrates a comparison of THz transmission and reflection imaging for the assessment of freshly excised and formalin-fixed, paraffin-embedded (FFPE) breast cancer tissue. First, THz imaging is utilized to define the three-dimensional boundaries of breast cancer tumors embedded in paraffin blocks. This procedure produces cross-section images of the tumor borders at depth and enables direct correlation with histopathology sections. Image processing of three-dimensional THz scans is implemented in order to develop automated imaging of breast tumors in a surgical setting.

In addition to FFPE tissue, this research develops tumor phantoms that match the THz properties of freshly excised breast cancer tissue. THz spectroscopy is used to develop phantoms based on optical properties reported in the literature for infiltrating ductal carcinoma. The phantom tissues are then used to construct complex models of excised breast tumors aiming to develop a practical application of THz imaging.

Finally, THz imaging is applied to fresh murine and human breast cancer tissue. Murine breast cancer tumors are generated via injection in mice maintained on a high-fat diet in order to closely model human breast tumors. On the other hand, freshly excised human breast cancer tissue is obtained from the National Disease Research Interchange (NDRI). The obtained THz images of fresh tissue are successfully compared to histopathology sections with good correlation.

Keywords: Biological Samples, Biomedical, Characterization, Imaging
Application Code: Biomedical
Methodology Code: Biospectroscopy
Recent practical advancements in THz source, detector, and system technology have enabled researchers to explore a myriad of medical applications in both research and research laboratory and clinical settings. While much progress has been made in clinically relevant investigations, clinical translation has been limited. In vivo, physiologic tissue does not display specific THz frequency spectral signatures and features identified in the excised tissue are often difficult to observe in vivo due to the large aqueous background. Model based analysis has also been limited as THz frequency electromagnetic models are highly sensitive to tissue morphology. In practice, physiologic variation is so broad that it is impossible to apply most models a priori.

In light of these issues are group has chosen to focus on three applications which we believe are ideal for THz diagnostic imaging technology. The first two are acute burn wound severity assessment and surgical flap viability assessment. In both cases, the immediate tissue response is characterized by massive changes in tissue water content (edema) allowing a THz based system to generate significant contrast without the need for substantial model based analysis or knowledge of tissue morphology.

The third application is the early detection of corneal diseases that are correlated with changes in corneal tissue water content. Current practice limits diagnostics to the measurement of corneal thickness and extrapolation to water content which itself is incredibly inaccurate and does not account for physiologic variation. While disease related changes in tissue water content are small, the physiologic variation in corneal thickness, as referenced to THz wavelengths is nearly 0. Thus the cornea can be treated as a thin film and model based analysis can be applied with reliable a priori assumptions on tissue morphology.

Keywords: Biological Samples, Biomedical, Biospectroscopy
Application Code: Biomedical
Methodology Code: Biospectroscopy
The ASTM AnIML projects provides a vendor-independent and technology-neutral data format for analytical data. This presentation starts with a summary of the current state of the AnIML project. Next, it explores how the format can be used to bring together data from multiple measurement techniques to enable higher-order analytics and collaboration. It concludes with a brief summary how BSSN Software uses AnIML in its products.
Analytical Information Markup Language (AnIML) Data Standard in Action

Analytical Data Longevity and Management for Regulatory Compliance Using AnIML Format and Cloud Technologies

In order to comply with FDA regulation (21 CFR Part 11), electronic records produced by Analytical instruments and Data Analysis applications are required to be stored for a very long and indefinite period of time. This poses significant challenges for the organizations as most of the instruments store data in proprietary formats. This requires to maintain a large number of potentially obsolete software and computer systems to be able to read that data, which is impractical.

Another significant challenge is a very large volume of data that is generated during the full cycle of Drug Discovery and Development. Data is processed and needs to be accessible by many individuals and parts of the organization often on global scale. It requires a system capable of managing all that information in a compliant manner.

Keywords: Laboratory Informatics, Liquid Chromatography/Mass Spectroscopy, Mass Spectrometry, Sample & Regulatory

Methodology Code: Laboratory Informatics
Previously we described the addition of support for storing, searching, and displaying mass spectrometry data in a relational database into ChemAxon’s Instant JChem (IJC) application, a desktop chemically-aware database client. This project has since been migrated to ChemAxon’s web-based Plexus Suite program, allowing for the same functionality of the desktop client using any modern web browser. Users can visualize the NIST/EPA/NIH Mass Spectral Library as well as custom GC/MS library files with associated structure and cheminformatics data. The MS data is stored in AnIML standard XML. A non-proprietary MS search algorithm has also been integrated into the application as well as support for LC/MS data types. Expanding this functionality to the web and integrating the AnIML standard into Plexus provides a powerful and flexible means to curate, search, display, and share both analytical and cheminformatics data with multiple users across many sites.

Keywords: GC-MS, Mass Spectrometry, Software
Application Code: General Interest
Methodology Code: Laboratory Informatics
Analytical Information Markup Language (AnIML) Data Standard in Action

AnIML Empowers Long-Term Storage and Archiving of GC and LC Raw and Meta Data

SCION Instrument’s CompassCDS is an industry-proven CDS (Chromatography Data System) that brings more than 20 years of legacy, continuous development and customer-experience to your desktop. Connected to a wide range of GC’s and LC’s from different vendors it provides a user-friendly, intuitive and application-rich user interface with extended calculation and reporting capabilities and industry- and function-specific add-ons and plug-ins to fit into any combination of laboratory and type of industry. Robust in design for 24/7 operations it scales from stand-alone to enterprise-wide client/server installations with centralized and secure system administration and data management. CompassCDS complies to national and international regulations and guidelines (21CFR11, ISO/IEC 17025, others) and is optimized for virtualized environments and metaframe rollouts. It seamlessly integrates into the existing IT infrastructure, and interfaces with LIMS, ERP or SCADA/PCS in order to facilitate, accelerate and automate data exchange, and helping to lower per sample costs and increase overall efficiency of the whole development and production process. Especially for Pharma and upcoming also for the Food industry high availability of archived raw and meta data and the possibility to review these data during audits without having the need to use the original software is a key requirement. Also for sharing of data within or across workgroups or research and development organizations and to finally overcome the limitation of pure PDF-based documentation for long-term archival CompassCDS LC and GC chromatogram data can now be reviewed in deep detail and also re-processed by using a unique AnIML-based viewer. At any time and without using the original software.

Keywords: Chromatography, Data Analysis, Laboratory Informatics, Software
Application Code: Pharmaceutical
Methodology Code: Laboratory Informatics
With the increased efficiency brought to labs by automated systems, the amount of data being collected, extracted, analyzed and manipulated is growing at an unprecedented rate. Likewise, the technology of automated devices, software platforms and information management systems is becoming more sophisticated to handle this surge in Big Data. However, further scientific advances and innovations critically depend on more complex, increasingly integrated, and highly adaptive systems.

In an effort to match these requirements, the lab automation’s information and communications technology industry has recognized the need for a flexible, modular approach to both the hardware and software of system integrations. However, modularity poses its own challenges. Laboratories still find themselves with a heterogeneous mix of different vendor hardware and software technologies, none of which easily lend themselves to seamless integration. In order for laboratory automation systems to adopt a modular, plug-and-play architecture, vendors must agree upon standards, both in the hardware as well as in the software, such that the system and the data the system produces can withstand reconfiguration and the test of time.

While over the years, several hardware standards have become adopted by laboratory automation device manufacturers (e.g. USB, Ethernet, SBS micro plate footprint), industry-wide software standards have been slower to emerge. The SiLA Consortium is uniquely focused on providing standardization solutions for both the horizontal communication between devices (e.g. status data, commands) and the vertical integration of result data and workflows. Join us to learn more about how SiLA satisfies the entire value proposition chain from device integration to data collection, transmission and analysis.

**Keywords:** Laboratory Automation, Laboratory Informatics, Sample & Data Management, Scientific Data Management

**Application Code:** Bioanalytical

**Methodology Code:** Laboratory Informatics
Electrochemistry at Nanoscale Structures

Electrochemically-Assisted Click Reaction for Spatially-Controlled Functionalization of Cylindrical/Conical Pores in Track-Etched Poly(ethylene terephthalate) Membranes

This presentation will report the spatially controlled modification of cylindrical/conical pores in a track-etched poly(ethylene terephthalate) (PET) membrane using diffusion-controlled, electrochemically-assisted copper (I)-catalyzed azide-alkyne cycloaddition (EC-CuAAC). A track-etched PET membrane was first modified with propargylamine via amidation of COOH groups on the pore surface, immersed in a solution containing Cu(II) and azide-tagged fluorescent dye, and then sandwiched between two electrodes. On the working electrode, Cu(II) in the solution was reduced to Cu(I) by applying an appropriate cathodic potential. The Cu(I) was expected to diffuse longitudinally along the pores, and then to catalyze CuAAC reaction of azide-tagged dyes with the ethynyl groups on pores. The pore functionalization was carried out by varying the duration of reduction step, and was assessed using fluorescence microscopy. So far, we have obtained the following results for 1-µm-diameter cylindrical pores: (1) The functionalization took place only at pore surfaces directly contacted to an underlying interdigitated working electrodes; (2) The amount of immobilized dyes increased at longer reduction step time; (3) Gradient functionalization was possible by controlling reduction step time; (4) The functionalization could be manipulated by the addition of hydrogen peroxide based on the re-oxidation of Cu(I). Longitudinally-controlled functionalization of a conical nanopore in a track-etched PET membrane using this approach will be explored, and will be assessed from ionic current rectification behavior. We believe the EC-CuAAC approach will provide a unique means for fabricating nanopores with spatial gradient.

This work is partly supported by the US-DOE (DE-SC0002362).

Keywords: Electrochemistry, Microscopy, Nanotechnology, Polymers & Plastics
Application Code: Material Science
Methodology Code: Electrochemistry
Electrochemistry at Nanoscale Structures

Microinjection into Microalgal Cells

The applications of vertically-protruding tapered microtube arrays (TMAs) are immensely diverse: they range from sensors, plasmonics, solar energy harvesting, battery electrodes, and transdermal drug delivery. Membrane-based template-synthesis methods are commonly applied as an inexpensive, generic platform to fabricate these three-dimensional tubes with submicron sized diameters. However, conventional membrane-based template approaches are limited in terms of preparing an array of short TMAs because the template membrane becomes increasingly fragile with decreasing membrane thickness.

In this presentation, we will describe a template-synthesis method that combines atomic layer deposition (ALD) and pulsed-current electrodeposition (PCD) to yield an array of tapered microtubes. The resulting TMAs have channels that are open on both ends, and the microtube heights that are not limited by the thickness of the template membrane. We will also demonstrate the potential application of this TMA as a microinjection platform for the widely-studied microalga [i]Chlamydomonas reinhardtii[/i]. Microalgae are a diverse group of aquatic, unicellular, photosynthetic organisms that are explored intensively for their ability to produce high-value-added products such as biofuels, recombinant proteins, pharmaceuticals, and fine chemicals. However, their hard cell wall has proven to be a critical barrier that hinders the advancement of microalgal technology. Our study offers novel opportunity to bypass the cell wall barrier and to extend the growing microtube-array-based injection technology from the widely-studied mammalian systems to the much smaller microalgal systems.

[b]Reference:[/b]

Keywords: Biofuels, Biotechnology, Electrochemistry, Material Science

Application Code: Material Science

Methodology Code: Electrochemistry
We present our advances in the measurements of photocatalytic activity of suspended nanoparticles of anatase TiO$_2$. We estimate the rate of photooxidation of methanol for colloidal nanoparticles from studies of the reaction products in the bulk colloidal suspension and from the stochastic behavior of the suspended nanoparticles. The colloidal properties were studied by dynamic light scattering and compared to the studies of product formation and of the stochastic behavior. We discuss the implications of the production of formaldehyde, the main reaction product and the time dependence of the rate of formaldehyde generation. The complimentary study of the stochastic behavior of the nanoparticles in the colloid is also discussed in terms of agglomeration of the nanoparticles and its effects on activity.

Keywords: Energy, Materials Characterization, Semiconductor
Application Code: Nanotechnology
Methodology Code: Electrochemistry
# Electrochemistry at Nanoscale Structures

## Abstract Title
Electroanalytical Characterization of Surface Properties and Reactivity of Engineered Nanoparticles by Collision Electrochemistry

### Co-Author(s)

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### Abstract Text
Nanoparticle collision electrochemistry provides extensive capabilities for detection and characterization of nanoparticles. This presentation will describe development of an electrochemical nano-impact technique for evaluating the fundamental surface properties, functionalization and reactivity of metal and metal oxide nanoparticles. The presentation will demonstrate the potential of this method for the: 1) assessment of surface reactivity of redox active nanoparticles, 2) monitoring surface adsorption/desorption and biorecognition events at single particle surfaces, and 3) as a method enabling rapid label free detection of biomolecular recognition targets. We will demonstrate capabilities of this method as a novel tool for assessing environmental contaminants, fate and transformation of nanoparticles in the environment, and for characterizing biomolecule-nanoparticle conjugates for biosensing design, analytical and diagnostic applications. Potential advantages and limitations of this approach as a method for the routine study of nanoparticles and nanoparticle systems will also be discussed.

### Keywords
- Bioanalytical
- Electrode Surfaces
- Environmental Analysis
- Material Science

### Application Code
- Environmental

### Methodology Code
- Electrochemistry
Electrochemistry at Nanoscale Structures

Stripping Analysis of Electrophoretically Deposited Nanoparticles and Nanoalloys

Citrate-coated Au Nanoparticles were deposited electrochemically onto indium-tin-oxide-coated glass electrodes in the presence of hydrogen peroxide by catalytic oxidation of peroxide. This results in the liberation of protons at the electrode surface, neutralizing the citrate stabilizer and causing the nanoparticles to deposit on the electrode surface. The most catalytic nanoparticles deposit on the surface because otherwise there is not enough protons generated to neutralize the nanoparticles. This leads to a big size-selectivity in the deposited nanoparticles, depending on the applied overpotential. Stripping analysis allows determination of the size and coverage of the deposited nanoparticles. Cu-Au and Cu-Ag alloy nanoparticles show interesting oxidative stripping behavior, depending on the electrolyte solution and the atomic arrangement of the atoms in the nanoalloy. Depending on the electrolyte used, the Cu and Au or Cu and Ag stripping peaks can be easily distinguished for quantitative analysis of the alloy composition. In certain electrolytes, new peaks appear in the voltammetry, indicative of the specific atomic arrangement in the nanoalloy and of ion adsorption at specific sites. The stripping analysis is also useful in probing atomic rearrangement in alloy nanoparticles that occurs during catalysis, heating, or other reactions.

Keywords: Electrode Surfaces, Nanotechnology, Stripping Analysis, Voltammetry
Application Code: Nanotechnology
Methodology Code: Electrochemistry
Electrochemistry at Nanoscale Structures

Controlling Electron Transfer in Nanoparticles and Protein Complexes

Electron transfer in nanometer-size chemical systems is an important process for the creation of macromolecular electronics. We are interested in the electrochemistry of metallic nanoparticles, redox proteins like Photosystem I, and other electroactive nanomaterials that bridge the gap between bulk solids and discrete molecules in the development of electronic devices using nanotechnology. In this talk I will discuss pH dependent switching on and off of electron transfer into nanoparticles, wiring nanoparticles and protein complexes for increasing electron transfer rates, and aspects of orientational control of electron transfer in these nanoscale systems.

Abstract Text

Electron transfer in nanometer-size chemical systems is an important process for the creation of macromolecular electronics. We are interested in the electrochemistry of metallic nanoparticles, redox proteins like Photosystem I, and other electroactive nanomaterials that bridge the gap between bulk solids and discrete molecules in the development of electronic devices using nanotechnology. In this talk I will discuss pH dependent switching on and off of electron transfer into nanoparticles, wiring nanoparticles and protein complexes for increasing electron transfer rates, and aspects of orientational control of electron transfer in these nanoscale systems.

Keywords: Electrochemistry, Nanotechnology

Application Code: Nanotechnology

Methodology Code: Electrochemistry
**Abstract Text**

Scanning electrochemical microscopy (SECM) has been used to study a wide variety of biological systems including bacterial biofilms. Herein, a new hydrogen peroxide sensor of sub-micro molar detection limit has been developed as an SECM probe to monitor the real-time microbial metabolic exchange between dual species biofilm. The dual species biofilm of [i] Streptococcus Gordon [/i] and [i] Streptococcus Mutans [/i] were immobilized at a known distance apart using our unique alginate-based hydrogel encapsulation technique. It was observed that the hydrogen peroxide producing capabilities of [i] S. gordonii [/i] were suppressed by the immediate presence of lactic acid producing [i] S. mutans[/i] biofilm. To correlate the hydrogen peroxide production with the change in biofilm microenvironment pH, a solid-state pH sensing SECM probe with near Nernstian slope has also been developed to map the pH above the gel-encapsulated dual biofilm. New findings of how the metabolites produced by these biofilms is affecting the immediate microenvironment including the biomaterials and vice versa would be presented at the meeting.

This work was supported by National Institute of Dental and Craniofacial Research (NIDCR), NIH (Grant # R21DE025370).

**Keywords:** Biomedical, Biosensors, Electrochemistry, Sensors

**Application Code:** Biomedical

**Methodology Code:** Electrochemistry
Nanoscale in-vivo studies on the release of a broad range of neurotransmitters are required to understand brain function and disease. Here, we demonstrate that the ion transfer across nano liquid-liquid interfaces supported on scanning electrochemical microscopy probes is a unique analytical platform for the study of neurotransmission on living biostructures. Typical nanoprobes are consisted of a laser pulled nano-pipette filled with an immiscible organic solution in the aqueous cell environment. Upon electrochemical polarization, the diffusion and transfer of charged neurotransmitters results in quantitative sensing and imaging. This versatile technique is capable of detecting species such as dopamine, acetylcholine, serotonin and tryptamine. (1)

We studied acetylcholine neurotransmission activity at single neuron structures from the model organism Aplysia californica. We employed scanning electrochemical microscopy (SECM) for accurate positioning of our nanoprobes on selected regions of cells and their synapses. By using the liquid/liquid approach, we circumvent challenges in the evaluation of redox inactive neurotransmitters using electrochemical methods. Our results show that our nanoprobes, with a typical diameter of 30 nm, can detect acetylcholine neurotransmission both qualitatively and quantitatively and in real time, with excellent signal to noise ratios and in biologically-relevant fluids. The strategy presented here suggests an exciting analytical pathway to study of the mechanisms of neurotransmission.


Acknowledgement: This research was supported by the National Institutes of Health under Award Number R21NS085665.
Commercialization and increased availability of Quantum Cascade Lasers (QCL) has enabled the mid-infrared sources to be used for laser absorption spectroscopy in a wide range of industries. The QCL presents many benefits to spectroscopic applications: high spectral power density, single mode emission, and a compact size that allows for system integration and stable operation in the lab and in the field. Combined with advancing MIR detector technologies, QCL-based systems offer high sensitivity in both gas and liquid phase sensing. As the technology matures, we will see the emission range of QCLs expand – today’s commercially available devices emit in the 4-12 µm range but in the near future manufacturers will tackle the terahertz region as well. What does this mean for existing applications, such as gas sensing and standoff detection? More importantly, what new applications are on the horizon? We will briefly cover existing QCL technology in rising applications as well as the hurdles left to overcome for future generations of QCL-based sensors in spectroscopy.

Keywords: Biomedical, Environmental/Air, Laser, Vibrational Spectroscopy
Application Code: Environmental
Methodology Code: Vibrational Spectroscopy
Mid-infrared spectroscopy is widely recognized as a powerful tool for protein characterization. The amide I band (~1700 – 1600 cm⁻¹) is particularly sensitive to protein secondary structure and can be used for protein quantitation and secondary structural analysis as well as an indicator of aggregation and amyloid sheets. Despite the power of the technique, measurement capabilities are typically limited to proteins at higher concentrations (> 5 mg/mL) and the experimental workflow can be tedious. In addition, due to the high absorbance of water, very narrow pathlength cells (~6-7 um) are required which further increases the difficulty of the measurement.

The high brightness of quantum cascade lasers shows promise in improving the sensitivity and usability of protein measurements (1). However, low frequency noise, environmental drift, and coherence related effects may limit performance. A microfluidic based sampling technique, referred to as Microfluidic Modulation Spectroscopy (MMS) has been developed to overcome these effects.

MMS allows for drift-free, accurate, high sensitivity absorption measurement with 10 to 100 times the performance of FTIR. Measurement workflow is greatly simplified due to microfluidic referencing, which minimizes interferences from atmospheric water vapor and temperature drift. The ability to control laser power enables linear operation over a dynamic range greater than four orders of magnitude. These capabilities are ideally suited to measurement of protein stability, quantitation, similarity, secondary structure, and protein aggregation. Examples for each of these characterization measurements will be reviewed in this presentation.


Keywords: Bioanalytical, Infrared and Raman, Protein, Vibrational Spectroscopy
Application Code: Bioanalytical
Methodology Code: Vibrational Spectroscopy
Quantum Cascade Lasers - A Different Approach to Infrared Spectroscopy?

Widely Tunable Quantum Cascade Lasers: Technology and Applications

External-cavity quantum cascade lasers (QCLs) are widely tunable sources of mid-infrared (MIR) radiation. They are ideally suited for a variety of chemical detection applications which require optical sources with high spectral brightness and high spatial brightness. These applications have, in turn, enabled the development of external-cavity QCLs that are rapidly tunable, reliable, rugged, and compact. QCL-based spectrometers are well suited for open-path atmospheric gas sensing for security and environmental monitoring applications. We will discuss the advantages of QCL-based spectrometers over Fourier-transform spectrometer (FTS) for this application and will present experimental results. For multi-component gas sensing, we demonstrate that widely tunable QCLs can be used for the quantification of the hydrocarbons C1 – C5 (methane through pentane) for mudlogging applications. For the detection of trace chemicals on surfaces, we present our recent work using active MIR hyperspectral imaging in which a target surface is illuminated by a tunable QCL and the reflected light is captured by a HgCdTe camera. Hyperspectral images with 128 x 128 pixels and >100 wavelengths are captured in <0.1 s. The hypercubes are analyzed to identify and spatially locate the surface chemicals (both liquids and solids). Widely tunable QCLs are shown to be an enabling technology for many important chemical detection applications.

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Keywords: Environmental, Infrared and Raman, Molecular Spectroscopy, Vibrational Spectroscopy

**Abstract Text**

Environmental, Infrared and Raman, Molecular Spectroscopy, Vibrational Spectroscopy

Application Code: Other

Methodology Code: Vibrational Spectroscopy
Quantum Cascade Lasers - A Different Approach to Infrared Spectroscopy?

Second Generation QCL Microscopy: Pushing the Limits of Infrared Chemical Imaging

The first commercial mid-infrared microscope using tunable quantum cascade lasers (QCLs) was introduced at the Pittsburgh conference in Chicago three years ago. Since its introduction, broad acceptance of the technology by the infrared spectroscopy community has demonstrated that QCLs are not only a viable source for demanding spectral imaging applications such as biochemical screening of tissues, cells, and fluids, but are likely to be the key to realizing the full potential of infrared chemical imaging. In addition to enabling new applications in biomedical and pharmaceutical research, the QCL microscope has provided a clear path to achieving clinical translation of automated, high-throughput infrared diagnostics.

The first-generation QCL microscope made possible for the first time, simultaneous wide-field with high-resolution infrared spectral imaging at video rates by leveraging newly available compound refractive optics and broadband uncooled focal plane array detectors. In some applications, throughput gains by as much as three orders of magnitude were achieved over conventional technologies while eliminating the need for cryogenic instrument cooling. However, despite these gains, demand for even higher throughput while maintaining high-spectral fidelity and low-noise performance has continued to motivate development in QCL instrumentation. In this talk, we will introduce the second generation of widefield imaging QCL microscopes, which leverages new rapid scanning, high-fidelity tunable QCL sources. The new microscope yields an additional enhancement in spectral image data collection speed by more than an order of magnitude while preserving the high spectral fidelity and SNR exhibited by the first generation QCL microscope. We will comment on the current state of QCL source technology and present a vibrant roadmap for infrared chemical imaging.

Keywords: Biospectroscopy, High Throughput Chemical Analysis, Infrared and Raman, Mass Spectrometry
Application Code: Pharmaceutical
Methodology Code: Vibrational Spectroscopy
Quantum Cascade Lasers - A Different Approach to Infrared Spectroscopy?

Discrete Frequency Infrared Imaging Using Quantum Cascade Lasers

Infrared spectroscopic imaging is a promising approach to biological tissue analysis that can reveal important molecular information without the need for substantial sample processing. In conjunction with classification algorithms, these instruments can provide objective and automated evaluations to aid pathologists improve diagnostic accuracy. Current industry standard spectroscopic imaging microscopes can take days or weeks to image a full tissue microarray. The quantum cascade laser, with higher intensity and narrowband emission, allows for a discreet frequency approach enabling drastic increases in imaging speeds while maintaining competitive spatial and spectral resolution. Here we present an instrument with new optimized optics minimizing aberrations and capable of diffraction limited performance at all fingerprint-region wavelengths across the entire field of view. We demonstrate high throughput imaging of tissue sections and tissue microarrays and evaluate the advantages in data quality obtained from a well-corrected system.

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Abstract Text
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Keywords: Instrumentation, Laser, Microspectroscopy, Vibrational Spectroscopy
Application Code: Biomedical
Methodology Code: Microscopy
Direct quantitative analysis of milk proteins (casein and lactoglobulin) in milk has been achieved in a 40 [micro]m flow cell using an external cavity quantum cascade laser which covered the spectral range from 1600 to 1700 cm\(^{-1}\). Furthermore, using the same completely room temperature operated set-up, results on protein folding in normal and heavy water will be shown as well. Concerning gas sensing a new approach based on three tone dispersion spectroscopy will be introduced on the example of CO sensing. The obtained results will be compared with those obtained using standard 2f wavelength modulation absorbance spectroscopy.

Keywords: Infrared and Raman, Laser, Process Control, Sensors
Application Code: General Interest
Methodology Code: Vibrational Spectroscopy
Quantum Cascade Lasers are spectrally bright light sources that have clear benefits for traditional spectroscopic applications and profound advantages in areas like trace gas detection. However, the coherent nature of these sources creates numerous challenges in microscopy and imaging applications. Traditional image sensor based approaches, either linear or 2D array, give rise to numerous coherence artifacts that significantly degrade image quality and impede quantitative interpretation. Laser Direct IR (LDIR) imaging is a fundamentally new Mid-IR imaging architecture that has been developed to overcome these limitations. LDIR retains the speed advantages inherent in a QCL imaging system and additionally enables the user, for the first time, to generate metrology grade images and spectra. An overview of LDIR will be presented along with quantitative measures of instrument performance along with numerous application examples.

Abstract Text

Keywords: Imaging, Infrared and Raman, Instrumentation, Vibrational Spectroscopy
Application Code: General Interest
Methodology Code: Surface Analysis/Imaging
Advancements in Environmental Monitoring

Open Source Instruments and Chemical Analysis Methods for a Citizen Science Based Environmental Monitoring Initiative

Citizen science is defined as scientific project conducted wholly, or in part, by amateur or non professional individuals. Simple ecological and environmental monitoring techniques such as performing bird censuses using volunteer birdwatchers have been in use for a long time. However, the more advanced instrumental analysis based environmental monitoring using the citizen science approach has become practical only in the past decade, after microcontroller (MCU) boards such as Arduino and Raspberry Pi substantially reduced barriers to entry by being both, low cost and user friendly; making them much more accessible to amateurs.

This talk discusses development of a modular and open-source UV-Vis spectrometer based on the latest MCU board with relevant details such as optics design, programming the boards, and its graphical user interface. We show how this spectrometer and other open source instruments can be used to quantitatively analyze chemicals such as pesticides, food contaminants etc using simplified analytical methods specifically designed for a citizen scientist. We will also introduce an integrated web platform, intended as a curated information portal for open source instrumentation, analytical methods, and a data sharing platform.

Keywords: Education, Environmental Analysis, Spectrometer, Wet Chemical Methods
Application Code: Environmental
Methodology Code: UV/VIS
Improved capabilities in microfluidics, electrochemistry, and portable assays have resulted in the development of a wide range of point-of-use sensors intended for environmental, medical, and agricultural detection in resource-limited environments in developing countries. However, these devices are generally developed without direct interaction with their often-remote intended user base, creating the potential for a disconnect between users’ actual needs and those perceived by the researchers developing the sensors. As different analytical techniques have inherent strengths and limitations, development of effective measurement solutions requires consideration of desired sensor attributes early in the development process.

In this work, we present our findings on design priorities for point-of-use bacteriological water sensors based on our fieldwork in rural India, as well as a guide to fieldwork methodologies for use in determining desired attributes for other point-of-use sensors. Based on our initial group workshops, we identified five sensor attributes of interest: affordable enough to be owned at household level, integrated output of amount of contaminant and recommended action, same-day or immediate results, reusability, and minimal required mixing of chemicals. Based on our conjoint analysis interviews, we evaluate the relative importance of each of these attributes. We present initial results in the development of a bacterial water sensor meeting these design priorities, as well as good practices for field-based determination of design priorities as a guide for other sensor developers.

Keywords: Environmental/Water, Sampling, Sensors, Water
Application Code: Environmental
Methodology Code: Sensors
Microcystins Release and Removal from Cyanobacteria During Oxidation Monitored by UFLC-MS/MS

Cyanobacteria in water source is a potential health risk. Microcystis aeruginosa (M. aeruginasa) is the most commonly reported species. In this study, different oxidants were applied to treat the M. aeruginasa in river water to investigate the release and removal of microcystin-LR (MC-LR).

An ultra-fast liquid chromatography tandem mass spectrometry (UFLC-MS/MS) method was optimized and validated to monitor cyanotoxin concentrations. Various amounts of free chlorine, permanganate, and peracetic acid were added into Missouri river water with different ammonia concentrations. At different reaction time, intracellular and extracellular MC-LR concentrations were monitored. The results showed that even very small amount of free chlorine can cause significant toxin release while greater amount were required for permanganate. Free chlorine was reactive with the extracellular MC-LR, but the monochloramine generated by the free chlorine and ammonia was not effective for MC-LR removal. Hence, in the water with high ammonia concentration, before free chlorine reaching breaking point, significant increase of extracellular MC-LR was observed. MC-LR, once released, could be rapidly oxidized by permanganate resulting in only negligible buildup of extracellular toxin. However, peracetic acid could not degrade MC-LR, resulting the toxin build up in water.

The authors acknowledge the Missouri Department of Natural Resources for funding this study.

Keywords: Environmental Analysis, HPLC, Mass Spectrometry
Application Code: Environmental
Methodology Code: Liquid Chromatography/Mass Spectrometry
Advancements in Environmental Monitoring

Towards an Automated Untargeted Method for Microcystins Analysis Using Two Dimensional Liquid Chromatography and Ion Mobility/Quadrupole Time of Flight Mass Spectrometry

Microcystins are cyclic heptatpeptide hepatotoxins produced by certain species of cyanobacteria (blue-green algae) found in freshwater environments. These secondary metabolites are toxic to higher organisms, causing human sickness or even death in some cases. Since few Microcystins are regulated and that there are only a handful of standards available, it is important to develop non-targeted methods for the analysis of these compounds. The present study describes an automated method for the analysis of microcystins by two dimensional liquid chromatography/quadrupole time of flight mass spectrometry (2D-LC/QTOF-MS).

An automated method for microcystins extraction and clean up from water samples was developed using 2D-LC in the trap and elute configuration was used: a large volume of water sample (500 µl) was directly injected and trapped in the first column. After that, microcystins were desorbed in reverse flow and injected to the analytical column, prior to mass spectrometry analysis.

The QTOF-MS was operated in high resolution full scan mode (RP 25.000) and proved to be very sensitive for microcystin-LR (50 fg on column with S/N > 10). Combined with the 2D-LC, the system could detect 100 pg/L of microcystin-LR in water with S/N>10. Mass accuracy (< 1 ppm) allowed assigning elemental compositions for unknown compounds with confidence. MS/MS mode monitoring the characteristic microcystin ion at m/z=135.0804 was useful to provide quantitative results of targeted compounds in complex samples.

More comprehensive characterization of complex algal bloom samples using ion mobility permitted the different microcystin variants to be distinguished. This new dimension of separation allowed the identification of some congeners that could not be separated by means of chromatography or mass spectrometry alone. Advanced acquisition methods were successfully employed to elucidate novel microcystin variants in authentic samples.

Environmental/Water, Liquid Chromatography/Mass Spectroscopy, Time of Flight MS, Water
Advancements in Environmental Monitoring

Measuring Ultrafines and Black Carbon - The Next Challenge in Air Quality

Toxicologists have determined that ultrafine particulates (<1um diameter) and black carbon are serious health risks, especially in emerging countries. Whereas PM 10 and PM 2.5 can be measured using laser scattering, these smaller particulates require other sizing technologies such as beta absorption (BAM) and electrometry.

We will present results for measuring ultrafines using photoionisation to charge the particulates, monitoring both the charged particulate and reactive ion currents. Modelling will be compared with lab and field data.

We will also discuss how this real time measurement relates to black carbon and whether electrometers can be used as a real time BC monitor in air quality networks.

Keywords: Environmental Analysis, Environmental/Air, Particle Size and Distribution, Portable Instruments

Application Code: Environmental

Methodology Code: Integrated Sensor Systems

Session Title: Advancements in Environmental Monitoring
Abstract Title: Measuring Ultrafines and Black Carbon - The Next Challenge in Air Quality
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Date: Tuesday, March 07, 2017 - Morning
Time: 10:05 AM
Room: W175a
Comprehensive two-dimensional gas chromatography (GCxGC) is gaining popularity in the analysis of environmental contaminants due to its ability to separate complex volatile and semi-volatile mixtures with increased peak capacity. The modulator, the "heart" of the a GCxGC system, is responsible for trapping, focusing and injecting the primary column effluent into the second dimension column. Modulators can be separated into two distinct categories: thermal and flow-based. A heater-based, thermal modulator traps the primary column effluent at or above ambient temperatures. A flow modulator utilizes pneumatic means to accomplish modulation of the primary column effluent by flow switching. A single-stage, consumable free thermal modulator and pneumatic flow modulator were compared. Both modulators were evaluated on their ability to perform trace environmental analysis of various sample matrices and obtain reproducible retention times. A BenchTOF-MS system was also implemented to determine detector responses and linearity over several orders of magnitude concentration range. For the differentiation of isomers and increase of S/N ratios, soft ionization was implemented. The results show the advantages and limitations of both thermal and flow modulation in the analysis of trace environmental contaminants. The designs of both modulators and the results obtained will be presented.

Keywords: Environmental Analysis, Gas Chromatography, GC-MS, Time of Flight MS
Application Code: Environmental
Methodology Code: Gas Chromatography/Mass Spectrometry
Advancements in Environmental Monitoring

Evaluation of a New Gas Chromatograph Incorporating a Novel Flow Path, Connection Technology, and Heating Approach for the Analysis of Semivolatile Organic Compounds

A new gas chromatograph (GC) has been developed which incorporates a novel modular flow path and connection technology to greatly improve usability by eliminating uncertainty in establishing and maintaining leak free connections. In addition, a new column format has been developed which requires about half the power in an instrument that is about half the size of the current state-of-the-art GC.

Gas chromatography/mass spectrometry (GC/MS) is widely regarded as the technique of choice for the measurement of semivolatile organic compounds (SVOCs) and is used routinely in environmental testing laboratories throughout the world. With the development of this new technology, it is critically important to show that the next generation of GC instrumentation can meet the rigors of SVOC analysis. The purpose of this paper is to demonstrate that this innovative GC can easily achieve the requirements of SVOC analysis as specified in USEPA method 8270D while maintaining equivalent retention time, resolution, and analyte response to current high performance GC/MS. In addition, the system durability over the course of repetitive injections of a soil extract containing a significant matrix load will be demonstrated. Results indicate that this new GC provides advantages for the analysis of SVOCs with respect to ease of use and resilience to matrix compared to current GC technology.

**Abstract Text**

A new gas chromatograph (GC) has been developed which incorporates a novel modular flow path and connection technology to greatly improve usability by eliminating uncertainty in establishing and maintaining leak free connections. In addition, a new column format has been developed which requires about half the power in an instrument that is about half the size of the current state-of-the-art GC.

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**Keywords:** Environmental Analysis, Environmental/Soils, Gas Chromatography/Mass Spectrometry, Semi-Volatile

**Application Code:** Environmental

**Methodology Code:** Gas Chromatography/Mass Spectrometry
Advancements in Environmental Monitoring

Practical Guidance to Increase Productivity, Reproducibility, and Efficiency with Microwave Extraction for Environmental Labs

Microwave Solvent Extraction (MSE) is an excellent addition to any contract environmental lab to compliment or replace existing soxhlet, sonication, or accelerated solvent extraction technologies. Due to MSE’s high-throughput capabilities (48 samples/hr) and low solvent consumption (20-30ml/sample), labs can achieve significantly higher productivity and drastically reduce operation costs.

EPA method 3546 for Microwave Extraction is applicable, to the extraction of semi-volatile organic compounds, pesticides, herbicides, phenols, PCB’s, and PCDD’s/PCDF’s. Closed-Vessel Microwave Extraction allows for higher temperatures to be reached, accelerating the rate of extraction with complete control of instrument parameters allowing for great reproducibility between runs.

Milestone’s Ethos UP for Microwave Extraction is utilized by a growing number of environmental labs. It will significantly increase productivity, reproducibility, and extraction efficiency while reducing everyday costs.

This presentation will focus on:

- Review of current extraction techniques
- Theory and Operation of Microwave Extraction technology
- Throughput comparisons with other extraction techniques
- Cost comparisons with other extraction techniques
- Review of EPA 3546

Presenter: Amit Joshi, Milestone Inc.

Ethos UP extraction on the web: http://www.milestonesci.com/#/extraction/j5t5b

Keywords: Environmental Analysis, Extraction, GC-MS, Microwave

Application Code: Environmental

Methodology Code: Sampling and Sample Preparation
When law enforcement, like Homeland Security is faced with a suspicious substance, the decision needs to be made if the substance should be sent to the lab for evaluation or if field testing with another method. High Pressure Liquid Chromatography (HPLC) is one testing method considered to be the definitive test to identify a suspicious compound for its presence and concentration. Because of the limited mobility and costs of traditional HPLC equipment, testing has required taking samples and sending the results to a laboratory, effectively meaning there are no real-time results in the field.

This study compares the efficiency, speed, and accuracy of portable chromatography equipment to laboratory equipment. The goal of the study was to see if portable chromatography could replace field testing with one device that could test for Cannabinols, Cocaine, and Amphetamine. Similarly the study can look into testing for presence of nitroaromatics and chemical weapons (CWA). Comparisons were made for ease of use, accuracy to discriminate the compound, accuracy of concentration measurement, and traceability with tracking reports.

Preliminary results show that the use of portable HPLC units, can detect and discriminate between the different drugs with accuracy levels comparable to lab based equipment. With RFID tagged sample probes and real-time generated reports, users were more confident in the traceability of their testing.

Keywords: Drugs, HPLC, Portable Instruments
Application Code: Homeland Security/Forensics
Methodology Code: Liquid Chromatography
Fentanyl is a synthetically produced opioid that, when produced and administered legitimately, is used to treat severe pain. Overseas labs in China are mass-producing fentanyl and fentanyl-related compounds and marketing them to drug trafficking groups in Mexico, Canada and the United States.1 Fentanyl traffickers have been successful at expanding their market and introducing new fentanyl-laced drug products in the United States.

GC-MS has been traditionally used for the analysis of pharmaceutical and “street” drugs; however, typical findings are often inconclusive due to high matrix effects which frequently include coelutions not evident in routine drug analysis results. In this study, a workflow was developed for the routine and comprehensive analyses of adulterated drugs. Sample preparation consisted of dissolving some quantities (1-5 mg) of sample in 1 mL of 8:1:1 chloroform, methanol, 1-butanol and sonicating for a couple of minutes. Sample solutions were transferred to a 2 mL GC vial and analyzed with a state-of-the-art, benchtop GC-TOFMS and modern software tools for data acquisition, effective processing and confident identification of compounds through spectral similarity searches using large, well-established databases. Baseline resolution of heroin and fentanyl were achieved in less than 3 minutes. Spectral similarity searches for samples with relatively large percentages of heroin (96%) and fentanyl (4%) were 855 and 887/1000 respectively. High performance analysis of adulterated drugs was achieved through rudimentary sample preparation, fast acquisition/processing times and confident identification of analytes using powerful software tools.


Keywords: Drugs, Forensics, GC-MS, Time of Flight MS

Application Code: Homeland Security/Forensics

Methodology Code: Gas Chromatography/Mass Spectrometry
Abstract Text

Synthetic drugs were first created to mimic the effects of traditional drugs such as THC; unfortunately, the chemical structures and physiological effects of synthetic drugs continue to change every year. Local and national agencies have begun to include synthetic drugs in their drug monitoring programs, but many of the tools and tests at their disposal are old, inadequate and often do not detect these novel synthetic compounds. Standard drug analysis techniques are riddled with shortcomings that thwart accurate and dependable characterization of synthetic drugs in complex matrices. This leads to misidentifications and compromised results due to heavy matrix effects which frequently include chromatographic coeluitions not apparent in routine drug analysis data. In this study, we conducted routine and comprehensive analyses of samples using a state-of-the-art, benchtop GC-TOFMS and modern software tools for data acquisition, effective processing and confident characterization of drugs through spectral similarity searches of large, well-established databases. A combination of electron/chemical ionization methods, high resolution GC-TOFMS mass spectrometry and novel software tools (e.g., deconvolution, mass defect, etc.) were utilized for the identification of unknown drugs.

Keywords: Forensics, Time of Flight MS
Application Code: Homeland Security/Forensics
Methodology Code: Gas Chromatography/Mass Spectrometry
Direct analysis in real time mass spectrometry (DART-MS) has gained widespread popularity in the forensic science and homeland security sectors. While it is an extremely useful technique it often lacks sample-to-sample reproducibility as well as difficulties in introducing large samples. The work will present several modifications that have been developed to combat these issues, allowing for the more repeatable and more sensitive analysis of larger pieces of materials containing forensically-relevant compounds such as narcotics, explosives (organic and inorganic), adulterants, and lotions and lubricants. Modifications which will be discussed include off-axis DART analysis, an independent thermal desorption unit for up to a 65% improvement in reproducibility and high level quantification, and the incorporation of acid chemistry for the detection of inorganic species. Methods for the controlled analysis of headspace will also be discussed.
Powerful inexpensive microcontrollers, electronic components, and 3-D printers are widely available. Electronic sensors and optical components are easily configured precisely with 3-D printed parts. Using these tools, undergraduate students in the chemistry laboratory are able to produce a wide range of instruments, including a visible spectrometer, potentiostat, pH meter, laser refractometer, and data logger. It will be demonstrated how these devices are constructed and controlled using open source software. Useful instrumentation does not need to be expensive. By having students build their instruments also removes the black box nature of these devices.
The Instrumental Analysis Laboratory Course at the University of Cincinnati underwent a course redesign including the revision and addition of laboratory experiments and expansion of lab report guidelines and materials. The Scientific Reasoning Tool (SRT) was developed as a way to scaffold the process of organizing information before writing a laboratory report. In addition, the tool was designed to address all of the course learning outcomes and support students in achieving these outcomes. The SRT is a one-page worksheet based on the Claim Evidence Reasoning model by McNeill in the form of a graphic organizer. McNeill’s model is a simplification of Toulmin’s Argument Pattern and was originally designed for grades 5-8, but has expanded to K-16 education. Two additional guiding questions were added which directly relate to sections of the laboratory report and provide the context for each experiment.

Student laboratory reports and SRT’s from the Inductively Coupled Plasma – Mass Spectrometry (ICP-MS) experiment in spring 2016 were evaluated for argumentation patterns and laboratory report quality. In addition, students were surveyed twice during the course to measure student attitudes and confidence in writing laboratory reports and using the SRT (if applicable). End-of-term Course Evaluations were also analyzed for key themes related to laboratory reports and the use of the SRT.

This work provides insight into current student writing abilities in the Instrumental Analysis Laboratory Course as well as their attitudes towards an intervention that was designed to improve their laboratory reports. The results from this work will be used to create additional materials and revise current materials for future iterations of the course. In addition, greater assessment training will be provided to Teaching Assistants prior to the course focusing on the use of the rubric and formative feedback.

Keywords: Education, ICP-MS
Application Code: General Interest
Methodology Code: Education/Teaching
What job can I get with a chemistry degree? This is a common question asked by students. Many analytical chemistry instructors do not have the resources or experience to answer this question. This presentation discusses a number of the strategies that we have developed at the University of Alberta to raise awareness of students to opportunities in analytical chemistry. For instance, we developed Introduction to Industrial Chemistry [1] as a means of introducing our students to the practices, environment, concepts, and other issues associated with the industrial workplace. The course has no traditional lectures. Rather it consists of seminars by chemists from local industry, industrial tours, informational interviews, and professional skills development such as resume writing and interview skills. Prior to these events, students watch short videos related to the career or industry [2], and post a reflection or question. As a final exam, students apply to a job description provided by a local chemist, and then do a “mock interview.” We have also developed a sophomore course that provides a low-stakes introduction to academic research. The outcomes of this early research experience will be discussed. The challenges and outcomes of both of these initiatives will be discussed. One of the more positive and least expected outcome has been the greater connection to our alumni.

1. www.ualberta.ca/chemistry/undergraduate-program/courses/chem-300
2. www.ualberta.ca/chemistry/undergraduate-program/resources-for-careers-in-chemistry
3. www.ualberta.ca/chemistry/undergraduate-program/courses/chem-299

Keywords: Education
Application Code: General Interest
Methodology Code: Education/Teaching
## Abstract Text

At present students gain practical experience from conducting laboratory classes in various disciplines. These sessions bring the science alive and usually underpin the theoretical content. However, unlike lectures, which have undergone a wide range of transformation due to the use of electronic technologies, laboratory classes have vaguely remained similar. With the availability of new electronic technology and software, there is scope for greater understanding of these sessions also to be gained. We have developed an e-lab script, which the students can visualise for given practical classes prior to attendance. The laboratory scripts have videos, links and additional information regarding the safety associated with the practice. Such scripts provide students with greater knowledge of the techniques that are carried out and the associated risks with the practical. We will showcase the impact such e-lab scripts have had on the student experience. Our believe is that the e-lab script gives students more confidence and understanding of the expectations of classes and builds on their technical skills. Our educational platform provides an innovative way to enhance practical classes.

**Keywords:** Education, Teaching/Education

**Application Code:** General Interest

**Methodology Code:** Education/Teaching
We seek measurement solutions to an open problem with potentially serious consequences for commercial nanotechnology and water purity. Manufacturers are increasingly integrating nanoparticles into consumer products which release these nanoparticles into environmental and drinking water. However, methods are lacking to quantitatively, sensitively, and routinely measure nanoparticles in complex media. Here, we explore the limits of optical microspectroscopy to do so, with the goal of developing measurement methods for point-of-use purification of water, environmental monitoring, and waste-water treatment.

We disperse metal and metal oxide nanoparticles with varying sizes into aqueous solution. Deposition and evaporation of microdroplets on carbon substrates can enable absolute measurements of nanoparticle concentration, and correlative optical and electron microspectroscopy of nanoparticles. Darkfield and hyperspectral darkfield microscopy reveal differences in the scattering of light from nanoparticles with varying size and material composition. A simple darkfield microscope with a halogen lamp and color camera distinguishes between gold and silver nanoparticles with diameters of \( \nabla 40 \) nm and \( \nabla 100 \) nm, and generic metal oxide nanoparticles, potentially enabling basic point-of-use characterization. Hyperspectral darkfield microscopy with dispersed wavelength detection shows the potential to discriminates between some metal oxide particles and resolves size differences of tens of nanometers in noble metals, in a format that is suitable for environmental monitoring and waste-water treatment with higher throughput than is currently possible. Scanning electron microscopy and energy-dispersive X-ray spectroscopy validate the optical measurements and define their capabilities and limits.

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Keywords: Contamination, Environmental/Water, Materials Characterization, Microspectroscopy
Application Code: Nanotechnology
Methodology Code: Microscopy
The design of efficacious, selective heterogeneous catalysts relies on the knowledge of the nature of active sites and reactive intermediates involved in the catalytic transformation. This is also true in the case of carbon dioxide reduction, an important scientific and technological problem. With the goal of furthering mechanistic understanding of a complex transformation that yields multiple products, we are employing surface enhanced Raman scattering (SERS) to image carbon dioxide photoreduction on individual Ag nanoparticles within a heterogeneous dispersion. The lack of ensemble-averaging is allowing us to detect fleeting intermediates in the adsorption and reduction processes. From kinetic trajectories of individual nanoparticles, different classes of nanoparticles will be cataloged: i) those with relatively weak adsorption of CO2, which are not suitable as catalysts. ii) those which adsorb CO2 permanently and are possibly poisoned and therefore are also not catalytically suitable, and iii) those that show the right characteristics of adsorption, followed by catalytic photoconversion to products like formaldehyde or methanol. The origin of such heterogeneities in adsorption and photoreduction behavior are being traced to differences in nanoparticle structure or surface composition, from which structure/activity relationships will be established. This single-nanoparticle approach can be utilized for obtaining molecular-level insights into a broad range of industrially and environmentally relevant catalytic transformations.

Keywords: Materials Characterization, Metals, Microscopy, Microspectroscopy

Application Code: Nanotechnology
Methodology Code: Microscopy
Second harmonic generation is widely used for the imaging of collagen and is particularly promising for imaging the disorder in stromal collagen commonly associated with several types of cancer, including ovarian. Polarization modulated SHG measurements can provide molecular orientation information. Near infra-red laser sources commonly used for SHG microscopy demonstrate superior penetration depth in biological systems where scattering can significantly attenuate signal. In the context of collagen imaging, polarization modulation measurements can be used to experimentally determine fibril orientation within the collagen fiber. Imaging biological systems presents an additional challenge, as scattering results in loss of polarization purity. In combination with the previously reported nonlinear optical stokes ellipsometry technique, a model for recovering polarization dependent observables as imaged through scattering media is presented. Detected signal is separated into a linear combination of purely polarized components and depolarized components represented as Mueller tensors linked by a depolarization parameter. Recovery of the purely polarized term yields the true molecular and ensemble response, allowing the measurement of local orientation in turbid environments through a polarization-dependent imaging technique. Funding is gratefully acknowledged from NIH grant numbers R01GM-103401 and R01GM-103910 from the NIGMS.
The brain involves billions of cells organized with specific functional properties into a large number of distinctive regions. In each brain region, the tissue is constructed by a large number of cell types: neurons, oligodendrocytes, astrocytes, microglia, and maintained by an extensive blood vessels network. The cells are organized into localized multicellular units, in the vast cortical cell layers. These regions are highly interconnected via axonal fibers, and the study of brain connectivity is the goal of the field called connectomics. In general, comprehensive mapping of an entire brain has not yet been achieved, and a much remains unknown about the brain’s structure. So it is evident, however, the importance of vibrational hyperspectral imaging and computational analysis to brain mapping. Infrared (IR) hyperspectral imaging provides multiplexed molecular specificity and morphologic details. Normally IR hyperspectral imaging systems are limited by a trade-off between acquisition time, spatial resolution, sample coverage, and signal-to-noise ratio (SNR). Here we report an image fusion method that significantly extends current capabilities of IR imaging by fusing a confocal microscopy data with a hyperspectral one, to generate an image with the high spatial and spectral resolution. The focus of our research is to develop a mapping technique using IR imaging and confocal microscopy combined with high-performance computing to quantify the cytoarchitectural in the brain. On the other hand, this study would open the door to a new way evolving as a fusion mapping.
A supervised learning approach for dynamic sampling (SLADS) is described to reduce confocal Raman imaging time. Conventional confocal Raman spectroscopic imaging requires the acquisition of a large number of individual Raman spectra. In order to achieve desired signal to noise ratio (SNR) the imaging process often requires a prolonged acquisition time, especially when additional laser exposure time is needed for samples showing low Raman signal response. Previously reported methods to increase Raman imaging speed often focuses on reducing the data acquisition time at each pixel, which includes heavy modification to conventional microscope system, such as utilizing an electron multiplying CCD (EMCCD) as detector for enhanced signal detection, or a digital micromirror device (DMD) for spectroscopic sparse sampling. The dynamic sampling Raman imaging uses two galvanometer mirrors to direct the laser beam onto any desired location of the sample, and sends the corresponding Raman signal into a conventional Raman spectroscope. It analyzes the signal acquired from a previous measurement, calculates the next most information-rich location to probe, and direct the laser beam to the location to probe for image reconstruction. Dynamic sampling Raman imaging of a typical 2-component system shows that this approach significantly reduces the number of data points needed for reconstructing a Raman image, thus improves imaging speed. The SLADS algorithm implemented herein is designed for single pixel measurements, and is applicable in other similar microscopy or spectroscopy systems to improve imaging speed, or to reduce sample exposure time.

Keywords: Sampling, Imaging, Microscopy, Raman Spectroscopy
Application Code: General Interest
Methodology Code: Microscopy
Applications of Microspectroscopy for Materials Characterization

A Membrane-Based Biosensor Platform for Measuring Ligand-Receptor Interactions

Highly selective, and sensitive biosensors are needed to study the interactions of new drug candidates with their target membrane bound receptors. Optical biosensors have commonly employed fluorescence detection via covalent labeling of drug candidates. Although conventional fluorescence approaches offer high sensitivity, they have drawbacks, such as the potential to sterically hinder normal ligand-receptor interactions. To address limitations of conventional fluorescence biosensors we are developing a new approach that combines confocal fluorescence microscopy with a novel biosensor architecture that utilizes a fluorescently-labeled synthetic lipid membrane, also called a black lipid membrane (BLM). The BLM serves as both a cell membrane mimic for the reconstitution of membrane-bound receptors, and as a sensor transduction element.

The sensor will measure ligand binding is based on the increase in membrane mass upon the binding of a ligand to its specific membrane-bound receptor, operating in a manner analogous to microcantilever sensors. BLMs have a high surface area to mass ratio, offering a large area for ligand capture while providing high sensitivity to changes in mass. As proof of principle of this sensor design, cholesterol was introduced to the BLM, resulting in increased rigidity of the membrane. The BLM was mechanically oscillated and fluorescence intensity was measured via confocal microscopy to directly observe the frequency of membrane oscillation. Cholesterol was shown to modulate oscillation frequency via changes in membrane rigidity. Further development of this sensor platform towards the incorporation of membrane-bound receptor proteins will be discussed.

Abstract Text

Fluorescence, Membrane, Microscopy, Sensors

Application Code: Bioanalytical
Methodology Code: Microscopy
Applications of Microspectroscopy for Materials Characterization

Microlens Based Optical Microscopy

Intensive efforts have been made to achieve optical imaging resolution by surpassing diffraction limits for the last decade. The negative index super-lens, hyper-lens, super-oscillation lens, and other super-resolution approaches have been proposed to overcome the diffraction limit but are limited due to multi-steps and tedious fabrication considerations. We report a super-resolution microscope for optical imaging which attains a resolution below 100 nm in the bright field and fluorescence imaging mode. This noninvasive microscopy utilizes borosilicate glass \((n \approx 1.53)\) and polydimethylsiloxane (PDMS, \(n \approx 1.46\)) microlenses to collect higher diffraction light from the specimen. The microlens and the object are in close proximity (separated by \(< \lambda\) where \(\lambda\) is wavelength of the excitation) allowing the capture of the both the propagated and evanescent light from the specimen. The produced super-resolution and enlarged images in the virtual plane are then magnified by a conventional/confocal microscope. We demonstrate that the microscope provides superior fluorescence imaging of the Focussed Ion-Beam (FIB) fabricated nanopatterns and self-assembled fluorospheres on Digital Versatile Disc (DVD) and Bluray grooves using low numerical aperture objectives. These patterns were resolved by microlenses using 10x (0.25 NA) and 20x (0.40 NA) objectives in a wide-field microscope with >4 times enhanced magnification and >3 times larger emission intensity over without microlens based imaging. Furthermore, we demonstrate super high resolution on biological samples namely diatoms (pores size of 100 nm) and fluorescently tagged cow pea mosaic virus \((\sim 50\text{nm diameter})\). Super high resolution scanning of sample was performed over a large surface of DVD and nanopatterns without destruction of the samples. Linear variation of fluorescence intensity \((100\text{nm fluorospheres})\) over the microlens surface is demonstrated using confocal microscope. Overall, microlens based nanoscopy is easy to fabricate and use, inexpensive, and does not require special requirements for sample illumination and data processing. It has potential applications in various fields of life-, bio-, and nano-sciences for imaging purpose.

Keywords: Super high resolution, microlens, fluorospheres, DVD and Blu-ray

Abstract Title

Applications of Microspectroscopy for Materials Characterization

Microlens Based Optical Microscopy

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Keywords:

Data Analysis, Fluorescence, Microscopy, Nanotechnology

Application Code:

Nanotechnology

Methodology Code:

Microscopy
A microscopy approach is developed for quantifying second harmonic generation (SHG) activity of powders that largely decouples linear and nonlinear optical interactions. Decoupling the linear and nonlinear optical effects provides a means to independently evaluate and optimize the role of each in crystal engineering efforts and facilitates direct comparisons between experimental and computational predictions of lattice hyperpolarizabilities. With this method repeated measurements using different particle sizes, it is possible to discriminate between phase matchable (PM) and non-phase matchable (NPM) materials from the SHG scaling with mean particle size. The presence of phase-matching, in which the refractive indices of the fundamental and doubled frequency are identical due to birefringence, can profoundly impact the net SHG intensity produced by a powder. The influence of phase-matching is also generally highly size-dependent. As such, the inherent spread of crystal sizes present within a typical powder sample can be nontrivial to include in the modeling and data inversion. In this current microscopy approach, using a focused fundamental beam places a controllable upper bound on the interaction length, given by the depth of field. Because measurements are performed on a per-particle basis, crystal size-dependent trends can be recovered from a single powdered sample. An analytical model that includes scattering losses of a focused Gaussian beam reliably predicted several experimental observations. Specifically, the measured scattering length for SHG was in excellent agreement with the value predicted based on the particle size distribution.

Keywords: Method Development, Microscopy, Process Analytical Chemistry, Quantitative

Application Code: Process Analytical Chemistry
Methodology Code: Microscopy
Use of Electrospinning and Dynamic Air Focusing to Create Three-Dimensional Cell Culture Scaffolds in Fluidic Devices

Organs-on-a-chip has emerged as a powerful tool. A fluidic approach for cell culture provides continuous nutrient supply and waste removal, gradient control and mimicking of in vitro microenvironments (i.e., shear stress). Most of currently reported Organs-on-a-chip models cultured cells on flat surfaces, which cannot represent the extracellular matrix (ECM) in vivo. Limited research has been done to integrate 3D scaffolds within a fluidic device. Electrospinning has been widely used to make fibrous scaffolds for cell culture. Although there are few reports showing the integration of electrospun fibers in fluidic devices, they needed complex techniques and procedures. Direct electrospinning of fibers into a sealed fluidic channel is not possible with the conventional setup due to the difficulty of focusing fibers into a small space. In this work, a dynamic focusing method was developed to directly electrospin fibers into a sealed fluidic channel, as a matrix for cell culture and subsequent studies under flow conditions. This method can generate fibrous layers on the inside a fluidic device (fiber size = 1.6 ± 0.6 µm and pore size = 113 ± 19 µm²). Cells were able to be cultured on this scaffold without using adhesion proteins. Macrophages cultured on fibers in a channel were found to secret significantly more cytokines than those cultured on a 2D surface in a channel. Microfluidic detection modules are also being studied, which can be coupled with the cell culture devices for near real time measurements.

Keywords: Bioanalytical, Biotechnology, Electrochemistry, Pharmaceutical
Application Code: Biomedical
Methodology Code: Microfluidics/Lab-on-a-Chip
Traumatic brain injury (mTBI) is a very common worldwide incident that can lead to a long-term physical, emotional, and behavioral disability. However, the lack of molecular marker based TBI diagnostics prevents improvement of the mTBI treatment. As a novel noninvasive biomarker, brain-derived exosomes have been found in the bloodstream after brain injury. These exosomes need extensive sample preparation (>24hr) for isolation due to their small sizes (30-200 nm). To address these challenges, we used a previously developed chip that can rapidly and specifically isolate subtypes of exosomes by making millions of magnetic nano pores working in parallel. (Fig. a) Using this chip, we profiled brain-derived exosomal microRNAs (miRNAs) in plasma samples from healthy and blast-induced injured mice using small RNA sequencing. (Fig. b) By identifying miRNAs that were statistically differentially expressed from both groups (Fig. c), we were able to diagnose TBI mice from healthy mice. (Fig. d)
A new platform for the PCR-free, label-free, optics-free sequence-specific detection of 16S rRNA from *Escherichia coli* at 1 aM has been demonstrated. Assays for nucleic acids of specific sequence are useful for the detection of pathogens in body fluids, food, or water. Most existing methods of detection are complex and require special reagents, such primers and polymerase for polymerase chain reaction (PCR) or fluorescent labels for optical detection. A glass nanopore in a thin glass membrane was fabricated as the basis of our new sensing device. The chip containing the glass membrane and nanopore was sandwiched between two, 200 [micro]L chambers milled from Teflon. An Ag/AgCl electrode is placed in each chamber such that a potential can be applied between them through the nanopore. Polystyrene beads conjugated with oligonucleotide capture probes composed of peptide nucleic acid (PNA) were added to one chamber of the device. In the absence of hybridized target nucleic acid, these bead conjugates are charge neutral and are not mobilized in an electric field. When complementary target 16S rRNA sequences, which are negatively charged, bind to the PNA probes on beads, the bead conjugates become electrophoretically mobile and move towards the pore in response to the applied electric field. The resulting pore blockage and drop in electrical current through the pore can be detected easily, thus giving a binary, yes/no method for diagnosing the presence of nucleic acid of specific sequence. We have demonstrated the ability of our nanopore device to specifically detect *E. coli* 16S rRNA at 1 aM against a background of 1 pM *P. putida* RNA. These results show that detecting small (>10 cfu) concentrations of pathogenic bacteria is possible with our device, even when the target organism is present against a large background of other bacteria.

Funding acknowledgements: NSF CBET – 1265061; NIH R21HG006157

**Keywords:** Detection, Nucleic Acids, Sensors

**Application Code:** Biomedical

**Methodology Code:** Sensors
A fully inkjet printed paper-based potentiometric ion sensing system consisting of an all-solid-state ion selective electrode (ISE) and a pseudo-reference electrode (p-RE) for sodium (Na\[^+\]) and potassium (K\[^+\]) ion measurements is presented. The patterned paper was fabricated by using a wax printer to define working and reference electrode zones. All other process steps of electrode fabrication were performed by using a piezoelectric Dimatix™ material printer (DMP-2800). Reference electrodes were fabricated by printing a PVC membrane containing a lipophilic salt, tetrabutylammonium tetrabutylborate (TBA-TBB), on top of an also inkjet-deposited pseudo Ag/AgCl reference electrode. Saturated KCl was immobilized in dry form on the top of the reference electrode to provide a stable potential and increase the stability of the electrode. A conducting polymer, poly(3,4-ethylenedioxythiophene) polystyrene sulfonate (PEDOT:PSS), was selected as working electrode material, and acted as both electric contact and ion-to-electrode transducer for potentiometric sensing. For specific ion measurements, polyvinyl chloride (PVC)-based ion selective membranes were coated on the PEDOT:PSS layer by the inkjet printer. These paper-based devices exhibited a stable potentiometric signal within 180 s, and required only small volume of sample (~25 µL). Sensitive response to Na\[^+\] and K\[^+\] ions with a linear range from 0.01 mM to 1.0 M and a detection limit lower than 80 µM was achieved for both ions. Moreover, this paper-based potentiometric approach provides good analytical performance, and shows outstanding performance for the direct, disposable and low-cost monitoring of sodium and potassium levels in biological samples such as human serum and urine.

**Keywords:** Ion Selective Electrodes, Paper/Pulp, Potentiometry, Sensors

**Application Code:** Biomedical

**Methodology Code:** Sensors
Contrast-enhanced computed tomography (CT) and spectral (color) X-ray CT have the potential to enable molecular imaging in CT. However, there is a lack of contrast agents designed to fully leverage the capabilities of spectral CT. Therefore, the objective of this study was to develop a modular approach to design a spectral library of core-shell nanoparticle (NPs) contrast agents, which will have broad applications in biomedical imaging due to potential for multi-modal imaging (e.g., fluorescence, MRI, X-ray, plasmonic resonance), dosed delivery of therapeutics and active targeting through molecular surface functionalization. Gd$_2$O$_3$, HfO$_2$ and Au core compositions were prepared at a common size (12-15 nm) using solution phase synthesis. Controlled silica shells (1-15 nm) enabled controlled loading of fluorescent molecules and provided a common platform for molecular surface functionalization. Antibodies and other small molecules were efficiently conjugated to the nanoparticles using EDC/NHS chemistry. The bioactivity and orientation of IgG antibodies conjugated to NPs were confirmed through agglomeration assays and electron microscopy. The multi-modal bioactive NPs were then successfully targeted to cd133(+) SKOV3-IP cells, which are responsible for poor prognosis in ovarian cancer patients and also HCC1954 (HER2+) cells, which are found in ~30% of breast cancer cases worldwide. Both cell lines were targeted with high specificity in vitro and intra-cellular distribution of NPs was detected using fluorescence microscopy. The binding kinetics of the NPs probes to the cells was also determined. Further, a xenograft model of ovarian cancer was constructed using cd133(+) SKOV3-IP cells in a nude mouse model. The tumor location was determined through fluorescence imaging and
Development of paper-based analytical devices (PADs) has become one of the most active research fields. Among a variety of detection motifs, colorimetry is preferably incorporated into (bio)chemical analysis using PADs for its ease of signal recognition. In comparison to quantitative colorimetry using camera and color analysis, visual inspection enables simple and fast chemical tests.

The present work describes the development of colorimetric PADs that display a semi-quantitative assay result in writing. Text-displayed PADs have been achieved by combining a colorimetric assay with a color-printed transparent film and a 3D-printed device housing. The printed color ink works as a screening color (SC), which screens the indicator color with weaker color intensity. Colorimetric indicators were inkjet-printed onto the PADs in the form of text that directly indicates the result of semi-quantitative assays. Although each text-shaped indicator shows the identical colorimetric response, its visibility is dependent on the SC to be overlaid. Users can semi-quantitatively determine the analyte concentration by reading out the text in the form of a visible maximum number on the PADs.

A colorimetric test for urinary protein has been selected, where the colorimetric indicator turns from yellow to blue with increasing concentration of albumin. The elaborated system allows semi-quantitative determination of urinary protein by reading the displayed symbols (negative, trace, +, 2+, 3+, 4+). The results of observer-dependent readout tests and the application to real sample analysis will also be discussed in the presentation.
Biomedical - Sensors, Nanotechnology and Microfluidics

Transparent Carbon Ultramicroelectrode Arrays (T-CUAs) as Sensors for the Detection of Pathogenic Cellular Response Mechanisms

Current methods for the detection of biogenic reactive oxygen species, like NO• and H2O2, include expensive, lengthy methods, notably fluorescence, chemiluminescence, and spectrophotometry. Reported is a new fabrication method for ultramicro- or nano- electrode arrays that overcomes typically lengthy processes, requiring many detailed assembly and processing steps that are sometimes unwieldy leading to poor reproducibility and repeatability of quantitative (electro)analytical measurements of biogenic species reactive oxygen species. Discussed is the facile fabrication technique for these array systems, and their figures of merit with respect to H2O2 as well as their spectroelectrochemical response. The modification and characterization of these transparent carbon ultramicroelectrode arrays (T-CUAs) as electroanalytical sensors for the in vitro detection of cellularly derived H2O2 and NO•, crucial physiological and pathophysiological molecules, are also discussed.

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Bioanalytical, Biosensors, Electrodes, Spectroelectrochemistry

Bioanalytical

Sensors
GC-MS are widely used analytical instruments. However, the vast majority of its applications are achieved with electron ionization, 30 m long capillary columns and 1 ml/min column flow rate. Consequently, most of the GC-MS performance parameters are similar and their improvements are not addressed.

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. 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 EI ion source and are mass analyzed by a single quadruple MS.

Several unexpected benefits of GC-MS with Cold EI are demonstrated:
A) We found by experiments and simulation that NIST library identification probabilities are improved with Cold EI as the molecular ions are the most sample characteristic ions.
B) Sample identification is superior to that of any other GC-MS since bigger and more labile compounds elute, exhibit molecular ions and elemental formula are provided with isotope abundance analysis software.
C) Significantly extended range of compounds are amenable for analysis via the use of short columns, high column flow rates and a contact-free fly-through ion source.
D) Compound independent ionization yield is provided.
E) Under 2 minutes analysis time is uniquely achieved with column flow programming.
F) The selectivity against matrix interference on the Cold EI molecular ion is as effective as MS-MS on a fragment ion.

Keywords: Gas Chromatography/Mass Spectrometry
Application Code: General Interest
Methodology Code: Gas Chromatography/Mass Spectrometry
Interest in the concept of field-deployable chemical detection technologies has continued to grow to a number of responding agencies. The transition of the extensive capabilities of traditionally laboratory-based systems, such as GC/MS, into outside-the-lab applications has been at the forefront of this concept for many years. While the output of transportable and fieldable GC/MS-based systems can approach the fidelity of lab systems, existing equipment trails the utility of lab-based systems due to compromises imparted by a challenging user interface, poor field readiness, and legacy pumping technology that limits practicality.

Recent innovations in down-range GC/MS instrumentation have addressed many of these pain points, creating a more field-viable, user-driven approach to chemical ID. The breadth of sample types has been demonstrated to be greatly enhanced through the adoption of an active, turbomolecular-based vacuum pumping system. By eliminating passive getter pumps, a standard liquid injector can be accommodated, making the down-range system able to accept direct organic liquid, SPME, solid, and vapor-phase samples, while eliminating the need for external service modules.

A further consideration of fielded systems is the expertise of the end user. Operators in this class are generally not GC/MS experts, necessitating a system with an easy-to-use interface, on-board reference material, and simplified field maintenance that improve the likelihood of successful operation in the field.

This presentation will focus primarily on the recent advancements associated with down-range GC/MS leading to the development of a truly portable system with the fidelity, utility, and practicality necessary for successful operation within field applications.
A Novel Benchtop GC-TOFMS for Improved Detection and Quantitation of Analytes in Complex Matrices

Introduction
Improved detection limits in GC-MS analyses are beneficial in a number of ways, including the detection of lower concentration analytes, a reduction in the amount of sample to be prepared for analysis, or the ability to use split injections to reduce matrix loading on the GC column and ion source. A novel benchtop GC-TOFMS with improved detection limit and wide linear dynamic range has been developed.

Methods
Replicate injections of multi-analyte standards were acquired on a novel, nominal resolution, benchtop GC-TOFMS to show detection limit and dynamic range capabilities on a variety of analytes. Instrument detection limits are calculated by
\[ \text{IDL} = \text{t}99\% \times \left( \frac{\text{RSD}}{100\%} \right) \times \text{amount on column}. \]

Results
The GC-TOFMS uses a novel direct extraction, open style ion source capable of generating very high ion currents, while keeping metal surfaces far away from the ionization region to reduce effects from matrix contamination. The robustness of the ion source will be demonstrated with a comparison of the instrument performance before and after hundreds of injections of complex matrix samples.
A novel, custom designed data acquisition system generates and transfers profile data to a state-of-the-art signal processing and data compression scheme in real-time during data acquisition. Combining the novel ion source optics and advanced data processing results in sub-picogram detection and quantitation of analytes present in complex matrix all while collecting full mass range spectra (m/z 10-1500) at up to 5 orders of linear dynamic range.

Keywords: Gas Chromatography/Mass Spectrometry, GC-MS, Instrumentation, Time of Flight MS
Application Code: General Interest
Methodology Code: Gas Chromatography/Mass Spectrometry
Homemade explosives (HMEs) present an ongoing threat to homeland security. There are a variety of different chemical classes of HMEs, of which binary mixtures, consisting of an oxidizer and a fuel, comprise a significant portion. The oxidizer and fuel used in a particular device can both vary, often dictated by the ease of acquiring the substances commercially. In particular, ammonium nitrate based explosives have become increasingly common both at home and abroad due to the ease of obtaining ammonium nitrate in a variety of forms. Explosive devices made from these materials present a challenge for detection due to the large number of possible oxidizer-fuel combinations as well as the near zero vapor pressure of ammonium nitrate.

Two vapor sampling methods were developed for the quantification of ammonia by GC/MS analysis. A passive sampling method, using solid phase microextraction (SPME), and an active sampling method, using an online cryotrapping inlet, were used to allow for different sampling techniques for different applications. Both methods utilized the derivatization of ammonia to butyl carbamate using butyl chloroformate to increase the trapping efficiency of the vapor sampling techniques as well as the detection capability of the mass spectrometer. Detection limits in the parts-per-billion range were obtained with both techniques. Additionally, research was demonstrated the ability to quantify ammonia from the dissociation of ammonium nitrate, while simultaneously characterizing the headspace profile of the fuel component of an HME mixture. By both quantifying the ammonia concentration while simultaneously characterizing the type of fuel present, additional information can be obtained about the nature of the threat.

Keywords: Derivatization, Gas Chromatography/Mass Spectrometry, Headspace, Sample Introduction
Application Code: Homeland Security/Forensics
Methodology Code: Gas Chromatography/Mass Spectrometry
The major strength of GC-MS in sample identification is its ability to perform library search for identification with names and structures. However, library identification cannot always be trusted and many compounds are not included in the library. Thus, we wish to be able to confirm or reject library identification and provide sample elemental formula from unit resolution GC-MS data.

We developed the Tal-Aviv Molecule Identifier (TAMI) software that automatically combines with the NIST library. TAMI inverts the molecular ion isotopomeric pattern and the measured mass (which it can improve its mass accuracy) into elemental formulae and automatically confirms or rejects library identification. In case of rejection it provides via a single click the sample elemental formula.

The TAMI software helps in: A) Improved mass accuracy of quadrupole mass analyzers to <50 ppm; B) Combines the improved mass accuracy of molecular ions with isotope abundance analysis for the provision of elemental formulae. TAMI begins with automated confirmation or rejection of NIST identification via isotope abundance analysis. In case of a confirmation the sample identification is unambiguous since it is done by an independent set of data and methods. Upon rejection, TAMI independently provides a table of elemental formulas with declining order of matching to the experimental data.

Thus, TAMI upgrades unit resolution quadrupole based GC-MS to be similar in sample identification capability to costly accurate mass GC-TOF-MS. Furthermore, TAMI operates with standard centroid data files and is easy to work with. You are invited to bring your files for its demonstration.

Keywords: Data Analysis, Gas Chromatography/Mass Spectrometry, Mass Spectrometry, Software

Application Code: General Interest
Methodology Code: Gas Chromatography/Mass Spectrometry
Volatile organic compounds (VOCs) emitted from bacterial cultures can reveal information on species and metabolism. As VOC concentrations are in the low ppbV range, pre-concentration techniques are required for GC-MS analyses. This study was intended to compare SPME and NTME for VOC analysis from cultures of Mycobacterium avium paratuberculosis (MAP).

For SPME, a 75 µm CAR/PDMS fibre was exposed to 20 ml headspace over cultures for 20 min at 37°C. For NTME, 20 ml were sampled bidirectionally through a needle packed with DVB, Carbopack X, and Carboxen 1000. Pure media samples served as controls. After thermal desorption VOCs were identified and calibrated by means of GC-MS in the range of 1 to 1000 ppbV by pure reference substances. 73 VOCs in the low ppbV range were identified as potential biomarkers. 36 VOCs were found with both methods. Most alcohols were assessed only by NTME. Limits of detection ranged from 0.09 to 69.52 ppbV (Median = 0.82 ppbV) for NTD and 0.06 to 74.54 ppbV (Median = 1.06 ppbV) for SPME. R² of calibrations with the NTD showed a mean of 0.995 (±0.019 SD) and 0.998 (±0.015 SD) for SPME. VOC patterns determined by both methods revealed differences between bacteria and pure media samples.

SPME and NTME were suitable for VOC preconcentration from bacterial cultures. Due to the principal modes of action (distribution vs. extraction) selectivity depends on physico-chemical properties of the substances. In VOC profiling, preconcentration techniques have to be chosen in accordance to the target biomarkers.

Keywords: Gas Chromatography/Mass Spectrometry, SPME, Trace Analysis, Volatile Organic Compounds
Application Code: Biomedical
Methodology Code: Gas Chromatography/Mass Spectrometry
Prostate cancer (PCa) is the second most common cause of male cancer specific mortality in the United States. Though early detection of PCa is critical to treating the disease, the lack of sensitivity and selectivity of prostate specific antigen (PSA) in PCa screening has stimulated an intense search for more reliable biomarkers of the disease. Recent studies have demonstrated that dogs can differentiate PCa patients from control negative by sniffing their urine. As the odor profiles are constituted by volatile organic compounds (VOCs), the aim of this study was to identify PCa-specific VOCs in urine for PCa diagnosis.

The study included 75 men who presented for trans-rectal ultrasound guided prostate biopsy for an elevated serum PSA (>2.5 ng/mL) or abnormal digital rectal exam. VOCs in urine were detected by Stir Bar Sorptive Extraction coupled with Thermal Desorption-Gas Chromatography/Mass Spectrometry. All VOCs were analyzed based on their occurrence and relative quantity in the urine. Potential PCa-specific VOCs were screened by two-sample t tests and Wilcoxon rank-sum tests. Furthermore, logistic regression was applied to develop models for potential VOC markers in PCa.

Of the 75 men, 35 were diagnosed with PCa with the remainder of PCa negative. A total of 6716 VOCs were detected in all urines. Statistical significance (at $\alpha=0.05$) in terms of the bivariate association with PCa prevalence was found in 36 VOCs that are related to PCa positive urine samples and 49 VOCs corresponding to PCa negative ones. Applying a liberal cutoff of 0.20 on the p-values, 263 potential VOCs were identified. After further selection with l1 regularization, the final logistic model involves 17 VOCs only. On the basis of predicted probabilities from the final model via cross-validation, the area under the receiver operating characteristic curve is 0.911, which indicates a highly promising discrimination power between VOCs in urine of PCa positive and negative patients.

The identification of a subset of VOCs that can reliably distinguish men with and without PCa highlights their role as potential biomarkers in PCa. Further validation with a larger sample size is warranted. Significant VOCs can be further investigated in the metabolomics study to understand their link to PCa.
Petroleum products are often complex mixtures that require significant time spent on data processing to make sense of, whether classifying hydrocarbon classes or determining the distribution of compounds with different functional groups. Just collecting the data may take one software, with processing it taking another, and then bringing that information into a visually pleasing plot or diagram that makes sense from a holistic point of view taking yet another different software package, all making it difficult to link the conclusions that characterize a sample back to original analytical data. With advances in ChromaTOF-HRT software, multi-dimensional chromatographic samples with high-resolution, accurate-mass spectra collected with either electron ionization (EI) or chemical ionization (CI) can be easily viewed with chromatograms, mass spectra, Kendrick mass defect plots, van Krevelen plots, and more all within one program. The ability to select a mass defect series based on C#, calculate probable formulas, and then elucidate structure by correlating back to the structured two-dimensional chromatogram with deconvoluted spectral comparison to commercially available libraries allows for quicker and more powerful assignment of peaks. Characterization of complex samples becomes simple, and visualizing the differences between samples can be done with side-by-side comparisons of commonly used plots, coded to highlight specific heteroatomic species of interest containing sulfur, nitrogen, or oxygen.

**Abstract Text**

**Keywords:** Data Analysis, Fuels\Energy\Petrochemical, Gas Chromatography/Mass Spectrometry, GC-MS

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

**Methodology Code:** Gas Chromatography/Mass Spectrometry
The use of ion chromatography with suppressed conductivity has been well accepted as a reliable method of quantitation for various water matrices. The suppression technology reduces the total background conductivity and enhances the conductivity for fully dissociated ionic analytes, resulting in improved detection limits. Since its invention, the suppression technology has been continuously optimized to minimize the band dispersion and baseline noise. The addition of the electrolytic suppression greatly improved the ease of use.

In this talk, we will present our latest work on the suppression technology to further improve the ease of use. We will discuss its design and different modes of operations. We will also show the utility of this new technology for various sample matrices.

Abstract Text

Keywords: Environmental/Water, Ion Chromatography, Liquid Chromatography, Trace Analysis
Application Code: Environmental
Methodology Code: Liquid Chromatography
Recent Advances in Suppressed Ion Chromatography Using Carbonate Eluents in Achieving Low Background and Noise Performance

In ion chromatographic analysis, the presence of dissolved carbon dioxide in the eluent impacts the background and, in turn, affects the chromatographic performance by lowering the absolute peak response and reproducibility of the retention time and peak response. The introduction of the eluent generator (EG) system and the continuously regenerated trap column (CR-TC) address the above limitations and an automated means for generating high purity eluent for IC is now possible. However, the presence of dissolved carbon dioxide gas in the sample is also a pathway for introducing carbon dioxide into the IC system. Use of a gas permeable tubing based consumable such as Dionex® CRD 300 is recommended for such applications to minimize the peak constituting to carbonate.

In the case of Carbonate / bicarbonate eluents, the product of suppression is carbonic acid which results in a greater than 10 fold higher background than hydroxide eluents and relatively high baseline noise. The removal of carbonate from the suppressed carbonic acid is feasible with the CRD by drawing out the carbon dioxide. A re-circulated stream of external base (high concentration) is supplied to the regenerant channel that converts the removed carbon dioxide to carbonate anion and prevents the carbon dioxide gas from returning back to the eluent channel. Another option is to use a vacuum pump that draws the carbon dioxide gas out of the regenerant channel. The above approaches, however, are cumbersome and require additional pumps, reagents and add to the operational complexity of the setup.

Here, we will present an alternative approach to the above process that does not require any external reagents or pumps. We will compare the performance of the conventional method with the new improved method for carbon dioxide removal.

Keywords: Environmental/Water, Ion Chromatography
Application Code: Environmental
Methodology Code: Liquid Chromatography
Optimizing LED-Based UV Absorption Detectors for On-Column Capillary Liquid Chromatography

Many traditional time-consuming and labor-intensive methods for collecting, transporting and preparing samples for analysis in a laboratory are being eliminated by the use of on-site portable instruments. This trend raises concerns of how accurate and reliable measurements in the field are. In the case of liquid chromatography (LC), UV-absorption detectors with standard light sources, such as mercury lamps, have been used for decades in the laboratory; however, they were never intended for use in the field, especially in miniature LC systems. Hence, there is a need for greatly improving this detector for field use. An LED light source has advantages of long life, excellent stability, low cost, low power consumption and the convenience of small size. In this work, 260 and 280 nm LED-based UV-absorption detectors were specifically designed for on-column detection in nano-flow LC. By precisely positioning two ball lenses, a band-pass filter, and a fixed slit in an integrated fixture that focused the light from the LED directly in the center of the 0.150 mm LC capillary column, excellent signal intensity, low noise and stable signal were observed. The results obtained using this new fixture were compared with previous results obtained using an LED holder with variable slit and optics that required tedious, manual adjustment of alignment.

Keywords: Capillary LC, Detection, Detector, UV-VIS Absorbance/Luminescence
Application Code: Environmental
Methodology Code: Liquid Chromatography
Superficially porous particle (SPP) LC columns are a popular tool in liquid chromatography. SPPs generate high efficiency at lower pressure, relative to their totally porous particle column counterparts. The current trend with SPPs is reducing particle size for further efficiency improvements. The higher efficiency can be used to speed up analyses or improve results by increasing resolution and sensitivity. However, to realize the full potential of SPPs, a compatible instrument must be used. This work will outline how to match an SPP column with an LC system, including integrated column ID and tracking, as well as properly coordinated column and instrument configurations. Using an SPP column that is specifically designed to work with your LC system can allow for high throughput and high quality data with consistent performance from batch to batch, keeping your laboratory operating at peak efficiency.
Retaining and separating small polar molecules with reversed-phase liquid chromatography is a challenging task. Alkyl phase LC columns, like C18, are a common starting point for LC method development. However, highly polar analytes have a low affinity for, and are poorly retained on, non-polar C18 stationary phases. Several techniques may be explored to retain these compounds, such as: adjusting the mobile phase pH when the analytes are ionizable, adding an ion pairing reagent to the mobile phase, or selecting a more appropriate column stationary phase for the analysis. The wide variety of stationary phase chemistries currently available on superficially porous particle columns can facilitate method development; several chemistries are well suited for troublesome polar analytes and can be used under 100% aqueous conditions without the risk of dewetting. Superficially porous particles are known for their ability to generate high efficiency with low back pressure. High efficiency can contribute to resolving closely eluting peaks, while low back pressure allows for flexibility with LC instrumentation. This work will demonstrate a logical, stepwise methodology to enable chemists to retain and separate their polar analytes with superficially porous particle columns.

Keywords: HPLC, HPLC Columns, Liquid Chromatography, Method Development
Application Code: High-Throughput Chemical Analysis
Methodology Code: Liquid Chromatography
Complex mixtures demand new methods of separation. In our work, we have been investigating an alternate paradigm in separation science. Instead of manipulating the chemistry of the mobile phase during the course of a separation, stationary phase chemistry is instead changed. Such stationary phase gradients have many advantages including the ability to impact and improve selectivity, separation times, and analyte detectability. Stationary phase gradients have been made on TLC plates and more recently on a PEEK-encased silica monolith using controlled rate infusion (CRI). The application of CRI involves flowing a solution containing a reactive organosilane through the monolith, generating a concentration gradient along the length of the column.

The focus of our most recent work is the creation of multi-component gradient stationary phase gradients on in-house synthesized silica monoliths encased in stainless steel. Cladding in stainless steel makes the monolith more robust and durable. We have seen improvements in peak shape compared to our recent publication with PEEK/PTFE cladding. Expanding on the single component gradient concept, multi-component gradients comprised of amine and phenyl functionalities are being prepared. We believe that two-component continuous gradients on one column will exhibit synergistic effects, compared to two consecutive columns connected in series. These stationary phase gradients have many applications, and are currently being adapted for the purpose of chelation chromatography for the separation of metals, along with reverse phase/hydrophilic interaction chromatography modes for weak acid/base separation.

Funding for this project is from NSF (CHE-1609449).
Hydrophilic Interaction Liquid Chromatography: Fundamental Investigation of Column Equilibration for Polar Siliceous and Zwitterionic Stationary Phases

HILIC chromatography is a unique system that involves a variety of molecular interactions including hydrophilic partitioning, polar, and ion-exchange interactions. There has been increased interest in this chromatographic mode given the complementary nature to reversed phase analysis and the innate compatibility of HILIC with mass spectrometry. Given the growing number of stationary phases, supports, and the complexity of the HILIC environment, there is a need to understand the interactions present to facilitate successful method development. This study builds upon the present accepted theories in HILIC chromatography to explore long equilibration times that hinder method development and reproducibility. Both polar siliceous based phases and zwitterionic chemistries are evaluated. This research characterizes a suppression of analyte ionization in MS that correlates with column equilibration in HILIC mode. This event is measureable by observation of ubiquitous adducts of diisooctylphthalate added to the mobile phase system in trace amounts. Specifically, this approach is demonstrated in the LC-TOF analysis of cathinones using a bare silica HILIC stationary phase. Based on this methodology, further study of HILIC equilibration has been conducted with the goals of systematic evaluation of the analysis conditions that drive HILIC equilibration and correlation of retention mechanisms to column equilibration. Information from this study is used to determine best practices to monitor and rapidly equilibrate HILIC stationary phases.

Keywords: Drugs, HPLC, Liquid Chromatography
Application Code: Other
Methodology Code: Liquid Chromatography
Chirality adds an additional layer of complexity when addressing the separation and isolation of materials. These mirror image forms of a compound are identical in most physical and chemical properties. Therefore, separating enantiomers has always been difficult. With the advancement of HPLC stationary phase technologies, a number of chiral stationary phases have been developed that show selectivity for a wide variety of chiral compounds. Many of these broadly applicable phases are coated polysaccharide based and are very versatile even though they are limited to simple solvent conditions and mild acidic and basic conditions (pH 2-8).

The applicability of polysaccharide columns can be expanded with immobilized polysaccharide phases. Traditional solvents compatible with typical polysaccharide columns are those that are familiar to reverse, normal, and polar organic separation modes (Hexane, Methanol, Ethanol, Acetonitrile, etc.). Immobilized polysaccharide columns do not have the limitations when strong solvents or additives are used (THF, DMSO, DCM, MTBE, Acetone, etc.). A recent addition to the polysaccharide HPLC column arsenal is a version that is stable up to pH 12. In this presentation, relevant chiral pharmaceutical compounds will be used as examples. The main focus will be on how changes in eluent components, column temperature, and pH can affect selectivity and retention in chiral separations.
Thiodipropionic Acid (TDPA) is a dicarboxylic acid that contains a sulfur group. It is used as an antioxidant in pharmaceutical, cosmetic and food products. A stability-indicating reversed-phase HPLC method was developed for the assay of TDPA and estimation of its related compounds. Chromatographic separation of TDPA and its related compounds was achieved by using a gradient elution at a flow rate of 1.0 mL/minute using ACE 5 (C18, 4.6x50 mm, 5um particle size) as the primary column and Phenomenex Prodigy(ODS(2), 4.6x50 mm, 5um particle size) as the equivalent column at 35°C. The mobile phase A consists of 5% acetonitrile and 95% 0.05% phosphate acid in water and the mobile phase B is acetonitrile. A UV detector at 215 nm was used to detect the analytes. The total run time for this method is 12 minutes. The new 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 TDPA related compounds (from TDPA and from each other) that are present in aged and stressed degraded samples under heat, light, base, acid and oxidation. This fast reversed phase HPLC method is ideal for QC labs to conduct routine test for the assay of TDPA and estimation of its related compounds.

Keywords: HPLC, Liquid Chromatography, Pharmaceutical, Validation
Application Code: Pharmaceutical
Methodology Code: Liquid Chromatography
HPLC columns with C18 bonded phases have become a popular choice for method development and should be part of the analytical tool kit, but C18 columns cannot accomplish every separation. Even when C18 provides adequate retention, it may not provide enough efficiency or selectivity. This talk will suggest preferred HPLC columns and conditions for a wide range of polar, nonpolar, large and small compounds. Initial sample screening with C18 columns is not wasted effort because retention values establish the chemical and physical nature of sample components; and in many cases, C18 will adequately perform the analysis. Unless previous experience with sample components has already disqualified C18 columns, the initial step should be to screen with a rugged C18 column using solvent gradients from water to acetonitrile with universal detection (low-wavelength UV or MS). A powerful and unique aspect of Reversed-Phase Chromatography (RPC) is that a wide range of solute polarities can be rapidly screened with simple binary gradients. Mobile phase pH should also be screened when acids or bases are present. If target compounds are both retained and well-resolved, RPC with C18 columns should be adequate. When targets are retained but poorly-resolved, aromatic or polar-modified alkyl phases should be screened to improve selectivity. If compounds elute near the void-volume regardless of RP conditions, they may be too polar for RP retention and require Hydrophilic Interaction Chromatography (HILIC). When adequate column retention has been established, variables can be optimized for final HPLC method conditions. Screening chromatograms will be shown, and how variables impact selectivity and efficiency in RP and HILIC modes will be described.

Keywords: Chromatography, HPLC, Liquid Chromatography, Pharmaceutical
Application Code: Pharmaceutical
Methodology Code: Liquid Chromatography
A reversed phase HPLC method was developed for the identification and assay of Praziquantel, Afoxolaner, Moxidectin, and BHT/BHA, and estimation of Praziquantel, Afoxolaner, and Moxidectin related compounds in topical spot-on finished products (Praziquantel 10.0% w/v, Afoxolaner 4.0% w/v, Moxidectin 2.5% w/v). Three different C18 columns (ACE 3, Thermo Hypersil, and TOSOH TSKGel) have been screened. All three columns provide good separation for three APIs. The TOSOH TSKGel C18 column was selected because it provides the best separation between Moxidectin and its 2-epimer. The three APIs were stressed at different condition to make sure the HPLC method can separate all major degradants. The wavelength of UV detection was also optimized in order to detect all three APIs using only one wavelength.

The final HPLC analysis is carried out using a TOSOH TSKGel ODS-100V C18 column (4.6x150 mm, 3 µm particle size) maintained at 35 °C with mobile phase A (0.05% v/v of phosphoric acid in water) and mobile phase B (acetonitrile). Analytes are separated by a gradient elution and detected at 225 nm for Praziquantel, Afoxolaner, Moxidectin, and BHT/BHA. The total run time of the method is 35 minutes. Afoxolaner and BHT/BHA are identified by matching their retention times in sample solution chromatogram to those in standard solution chromatogram. Praziquantel, Moxidectin and related compounds are identified by matching their relative retention times vs. Afoxolaner to those listed in the method, respectively. Individual peaks for APIs are quantitated by the external standard method against the Afoxolaner peak in the working standard solution. Related compounds are quantitated by comparing their peak area to the corresponding API peak area in the same sample injection. BHT/BHA is quantitated against the BHT/BHA peak in the working standard solution. The reporting limit for Praziquantel, Afoxolaner, and Moxidectin related compounds is 0.3%.

**Keywords:** HPLC, Identification, Method Development, Pharmaceutical

**Application Code:** Pharmaceutical

**Methodology Code:** Liquid Chromatography
Solid-phase extraction (SPE) is a technique that is widely used in laboratories to isolate the analytes of interest from complex matrices. SPE is typically performed off-line and often requires significant manual effort. The off-line SPE processes are time consuming, labor intensive, difficult to automate, and often result in low reproducibility. Therefore, there is a trend toward the development of on-line SPE processes.

The presentation describes the recent development of on-line SPE cartridges with two reversed-phase chemistries, C8 and RP-Amide. The on-line SPE cartridges were applied to the LC/MS analysis of three thyroids including 3,3',5-triiodo-L-thyronine (T3), 3,3',5-triiodo-L-thyronine (rT3), and 3,3',5,5'-tetraiodo-L-thyronine (T4), which were spiked in human serum. The results show significantly greater (>25%) LC/MS response of all three thyroids with the on-line C8 or RPA cartridges comparing the analysis without the cartridges. Interestingly, the RPA cartridge leads to higher response than the C8 cartridges under the same conditions. The reproducibility (relative standard deviation, RSD) of the LC/MS signals from 120 consecutive injections of 100 ng/mL of each thyroid spiked in human serum is 5.5%-9.1% and 4.7%-8.8% with C8 and RPA cartridges, respectively. The applicability of the on-line SPE with LC/MS is also demonstrated with cannabis analytes in human plasma and plant extract, as well as drugs and their metabolites in urine samples. The above studies show the C8 and RPA cartridges enable on-line sample cleanup, concentration, and separation with excellent reproducibility and chromatographic performance. The technology promises to reduce time-consuming manual sample preparation and improve robustness and reliability of analytical methods.

**Keywords:** Liquid Chromatography/Mass Spectroscopy, Solid Phase Extraction

**Application Code:** Pharmaceutical

**Methodology Code:** Liquid Chromatography
In the first week or two following a traumatic brain injury (TBI) the brain often experiences secondary injuries, correlating with a poor patient outcome. During these events there is a depolarization of neurons that propagates like a wave across the cortex, resulting in an increase in extracellular potassium and an increased energy demand to repolarize the cells.

Brain microdialysis is an ideal method for detecting the chemical changes that occur during spreading depolarization (SD) waves. However, long-term microdialysis sampling suffers from ischemia and glial scaring which form around the microdialysis probe implantation site, thus hindering the ability of molecules to diffuse into the probe. Our lab has previously shown that adding dexamethasone to the microdialysis perfusion fluid (dexamethasone-enhanced microdialysis) significantly improves the ability to sample evoked dopamine responses 5 days after probe insertion. The goal of this project is to apply dexamethasone-enhanced microdialysis to improve the detection of SD waves in the 10 days following probe insertion. Glucose and potassium were measured with the rapid sampling microdialysis (rsMD) detection system developed by the Boutelle lab. Induced SD waves were monitored in the rat cortex 2 hours, 5 days, and 10 days after probe insertion. In comparison to controls dexamethasone significantly improved the sampling, providing detectable responses after 10 days.

Next, we will evaluate the long-term capacity for in vivo monitoring using dexamethasone-enhanced microdialysis in rats subjected to controlled cortical impact. With this TBI model, we will also monitor for naturally occurring SD waves in the rat cortex over several days.

Keywords: Bioanalytical, Biosensors, Electrochemistry, Neurochemistry
Application Code: Neurochemistry
Methodology Code: Sampling and Sample Preparation
Spreading depolarizations (SD) are neurological phenomena occurring during pathological states such as stroke, traumatic brain injury, and migraine. SDs are thought to play a major role in increasing the mass of dead tissue following an initial brain damage event, as they emanate and travel out from the core of the injury. SDs are characterized by a breakdown of ionic gradients across the cell membrane and subsequent release of neurotransmitters. In healthy tissue, an increase in cerebral blood flow follows this wide-spread depolarization of cells. This serves to bring in oxygen and glucose to provide energy to reestablish the disrupted ion gradients and remove excitotoxic compounds in the extracellular space.

A common approach for investigating SDs incorporates the measurement of oxygen changes. However, most of these measurements are restricted to cortical layers of the brain due the invasive and fragile nature of the sensors. Here, we use Fast-Scan Cyclic Voltammetry (FSCV) at carbon fiber microelectrodes to monitor simultaneous changes in oxygen, electroactive neurotransmitters, and electrophysiology in deeper brain tissues as well as the cortex. We have implemented these sensors to record oxygen and dopamine changes occurring during SD in the nucleus accumbens and compared it to responses recorded in the cortex.

Keywords: Electrochemistry, Neurochemistry, Voltammetry
Application Code: Neurochemistry
Methodology Code: Electrochemistry
Adenosine acts as both a neuromodulator and a neuroprotective agent in the brain. Transient adenosine release has been characterized by the Venton lab over the last 5 years in vivo, and has recently begun to be characterized in situ as well. In situ studies complement the work done in vivo because some pharmacological studies are not feasible in live rats due to issues such as toxicity or blood-brain barrier permeability. Initial comparisons to in vivo results indicate that adenosine transients in brain slices occur with similar frequencies and concentrations. However, transients do differ in frequency and concentration between different brain regions. Transient adenosine events in the prefrontal cortex and hippocampus occur every 15 seconds on average, while in the thalamus they occur every 2 minutes. Transients are significantly higher in average concentration in the hippocampus (0.24 M) compared to the prefrontal cortex (0.16 M) and thalamus (0.11 M). Lastly, we explored potential release mechanisms of transient adenosine events. The A1 receptor is blocked with the antagonist DPCPX, increasing the concentration of adenosine transients in the thalamus and hippocampus. Blocking pannexin 1 channels with spironolactone causes a dramatic decrease in the frequency of transients in the hippocampus. We also blocked sodium channels with TTX to determine if these events are activity dependent. Exploring these pathways will help elucidate the mechanisms by which adenosine serves as a neuroprotective agent.
Oxidative stress has been implicated as a key player in various neuropathologies such as Parkinson’s disease and Alzheimer’s disease. A variety of cellular processes are involved in the generation and breakdown of reactive oxygen species, such as hydrogen peroxide (H\(_2\)O\(_2\)), in the striatum; however, the extent to which each of these sources contributes to extracellular H\(_2\)O\(_2\) dynamics in striatal tissue remains unknown. Potential sources include mitochondrial activity, and the biosynthesis and metabolism of dopamine (DA). The goal of this project is to quantitatively investigate key contributors to striatal H\(_2\)O\(_2\) fluctuations using fast-scan cyclic voltammetry coupled to carbon-fiber microelectrodes. Pharmacological agents are used to manipulate (1) DA synthesis (L-DOPA, 100 mg/kg), (2) DA metabolism (pargyline, 150 mg/kg), and (3) H\(_2\)O\(_2\) breakdown (local infusion of mercaptosuccinate, 15 µg). Striatal H\(_2\)O\(_2\) dynamics are voltammetrically quantified in real time using a dual microelectrode device. This device consists of two carbon fiber microelectrodes, one of which is coated with an m-phenylenediamine (mPD) membrane - a size exclusion membrane that enables selective detection of H\(_2\)O\(_2\). The uncoated microelectrode is used to simultaneously monitor the effects of these drugs on local dopamine dynamics. The results indicate that each of these processes are contributing to the generation and breakdown of extracellular H\(_2\)O\(_2\) in the striatum to varying extents. Overall, this work sheds new light light on the pathways that contribute to oxidative stress in this critical brain region.

Keywords: Bioanalytical, Electrochemistry, Neurochemistry, Voltammetry

Application Code: Neurochemistry

Methodology Code: Electrochemistry
Solid phase microextraction (SPME) is a useful sample preparation tool that has demonstrated its suitability in the investigation of different biological systems. The small size of the sampling probes and the availability of biocompatible SPME coatings make of this technology an attractive approach for in vivo determinations. Taking advantage of such features, in this study we present an application of SPME to monitor in vivo the metabolic changes occurring in rat brains before and after deep brain stimulation (DBS). For this purpose, 4 mm mixed mode and C18 fibres were placed in the rat brains for 30 min with the help of a surgically implanted cannula and a positioner. The animals were subjected to 3 hours of DBS and the sampling was conducted before and after the procedure. Considering that with SPME non-lethal sampling is possible, multiple samplings from the same animal were carried out. All the collected fibres were desorbed in appropriate solvents and the extracts were analyzed using liquid chromatography coupled to high resolution mass spectrometry. Data processing was carried out using XCMS online and non-parametric tests. Several metabolites showed statistical changes when comparing extracts obtained before and after DBS. Glutamic acid, for example, was found to be up-regulated after DBS, which is in agreement with findings reported by other authors. Other compounds that exhibited statistical changes after DBS were citrulline, threonine, carnitine, lysine, phenylalanine and methionine (p<0.01).

Keywords: Neurochemistry, Sample Preparation, Metabolomics, Metabonomics

Application Code: Neurochemistry

Methodology Code: Sampling and Sample Preparation
New Approaches to Understanding Brain Function

Analyzing Single Vesicles in PC12 Cells Using Novel Pt Nanoelectrodes

Constant potential amperometry in which an electrode is held at a potential sufficient for oxidizing an electroactive species is a major tool for studying single-cell exocytosis. One of the current challenges for electroanalysis at single cells lies in spatial resolution. Currently, carbon fiber microelectrodes (CFEs) having diameters ranging from 5-10 µm are employed for single-cell amperometry experiments. However, CFEs are typically equivalent in size to the cells studied, and no further spatial information can be obtained at a sub-cellular level.

To improve spatial resolution in single-cell analysis, our lab has developed novel Pt nanoelectrodes based on focused ion beam (FIB)-induced metal deposition. An FIB-milled nanopore is first created near the tip of a sealed quartz nanopipette, into which Pt is deposited to form a 500 x 500 nm bipolar electrode. Electrochemical measurements can be made on the Pt nanoelectrode simply by filling the quartz nanopipette with an electrolyte solution containing a redox mediator, such as H⁺ or Ru(NH₃)₆³⁺. The process is easily reproduced, and could be continually miniaturized for the future application of recording from single neuronal synapses.

In this presentation, I will first describe the development and characterization of microfabricated Pt nanoelectrodes. Then I will discuss promising exocytosis results collected from dopaminergic rat pheochromocytoma (PC12) cells. Exocytosis is triggered by injection of a high K⁺ solution, releasing dopamine to the electrode surface. This work was supported with NIH (GM101133).

Keywords: Bioanalytical, Electrochemistry, Electrodes, Neurochemistry
Application Code: Neurochemistry
Methodology Code: Electrochemistry
To meet the increasing demand of a growing population and shrinking total arable land, the agricultural industry relies heavily on intense pesticide regimens. Many factors affect the potential harm a pesticide could have on a person’s health such as selectivity, mode of action and half-life in both water and soil. Exposures are common but rarely reported, primarily because people are often not aware they have been exposed. Education about the signs and symptoms of exposure is lacking and generally pertains only to acute, large doses.

Some of the most commonly used pesticides function in pests by inhibiting chemical breakdown in a synapse (enzyme inhibition) or modulating ion channels (blocking; reversing) causing a detrimental effect on the nervous system of the organism. It is not known whether pesticide exposure in mammals affects neurotransmission. In this work, existing and novel voltammetric tools are described to monitor neurotransmitters in mice that are exposed acutely and chronically to common pesticides. Dose response curves are constructed and the effects of varying doses on animal behavior are assessed. This study thus constitutes an important analysis of pesticides’ neurological effects.

**Abstract Text**

To meet the increasing demand of a growing population and shrinking total arable land, the agricultural industry relies heavily on intense pesticide regimens. Many factors affect the potential harm a pesticide could have on a person’s health such as selectivity, mode of action and half-life in both water and soil. Exposures are common but rarely reported, primarily because people are often not aware they have been exposed. Education about the signs and symptoms of exposure is lacking and generally pertains only to acute, large doses.

Some of the most commonly used pesticides function in pests by inhibiting chemical breakdown in a synapse (enzyme inhibition) or modulating ion channels (blocking; reversing) causing a detrimental effect on the nervous system of the organism. It is not known whether pesticide exposure in mammals affects neurotransmission. In this work, existing and novel voltammetric tools are described to monitor neurotransmitters in mice that are exposed acutely and chronically to common pesticides. Dose response curves are constructed and the effects of varying doses on animal behavior are assessed. This study thus constitutes an important analysis of pesticides’ neurological effects.

**Keywords:** Electrochemistry, Neurochemistry, Pesticides, Voltammetry

**Application Code:** Neurochemistry

**Methodology Code:** Electrochemistry
Prader Willi Syndrome (PWS) is the most commonly known genetic cause of life-threatening obesity. The major medical concern for patients with PWS is morbid obesity. Compulsive eating and obsession with food usually begins between the ages of 1 and 6. The urge to eat is physiological and overwhelming; it is difficult to control and requires constant vigilance. Weight control depends on food restriction and daily exercise. Currently there is no medication or surgical intervention that can eliminate the need for strict dieting and supervision around food. Studies have repeatedly shown that PWS patients are at risk of premature death. Mortality in children is most commonly associated with respiratory infection and high temperature resulting in sudden death. Common in adults with PWS are obesity-related problems such as respiratory failure and pulmonary hypertension, obstructive sleep apnea, hypertension, and type 2 diabetes mellitus (T2DM). The cause of death in adults is usually related to failures of the circulatory or respiratory systems.

BSN272 is a New Molecular Entity (NME) recently submitted to FDA for treatment of Prader Willi syndrome. The first batch of BSN272 successfully underwent toxicology testing and preclinical efficacy testing, and human trials are about to begin. In preclinical trials BSN272 appears to reduce both the craving for food and obesity. The first batch prepared for phase 1 clinical trials underwent spectroscopic characterization including ultraviolet and visible absorption spectrophotometry, infrared absorption spectrophotometry, specific optical rotation, mass spectrometry, and NMR spectrometry. Scanning electron microscopy of particles was also performed. The results of these studies explain the pseudo-polymorphism observed with the compound.

Keywords: Characterization, Clinical/Toxicology, Toxicology, UV-VIS Absorbance/Luminescence
Application Code: Pharmaceutical
Methodology Code: Vibrational Spectroscopy
Biopharmaceutical companies currently assess monoclonal antibody (mAb) integrity by using a wide range of analytical techniques, each capable of detecting a subset of molecular attributes. These methods, which are commonly based on electrophoresis, chromatography and spectroscopy, provide information about the size, charge and glycosylation profile of mAb drugs. Changes due to post-translational modifications during the drug development are also detected using these techniques. Although a wealth of information is obtained from the use of these analytical techniques during characterization, the biological activity of the molecule is ultimately considered the principal reflection of overall mAb structural integrity under physiological conditions.

Recently, a new IgG receptor, human Fc receptor-like 5 (FCRL5), sensitive to the whole IgG molecule, has been identified. Using SPR (Biacore) technology, it was established that FCRL5 binds human IgG via a complex mechanism, where IgG isotype is just one determinant of the interaction. Nevertheless, the full extent of molecular attributes that affect the human IgG-FCRL5 interaction remains unclear. Our hypothesis is that small structural changes in IgG alter FCRL5 binding, therefore assessing the interaction could be used to differentiate among very similar IgG molecules including biosimilars.

This work examines the progress being made in the development of a rapid and sensitive Biacore method to assess the integrity of therapeutic IgG2 mAbs. The assay is based on measuring the interaction of the therapeutic mAb with FCRL5, which requires an intact IgG molecule for high affinity binding. Changes in IgG2-FCRL5 binding observed using stressed IgG2 samples will be discussed.

Keywords: Biopharmaceutical, Biosensors, HPLC, Statistical Data Analysis
Application Code: Pharmaceutical
Methodology Code: Sensors
Fluid imaging techniques allow characterization of visible and sub-visible particles based on the morphology, but they lack compositional information. Raman compositional analysis is often performed on particles after they are isolated from a sample, typically by filtration. Filtration can alter protein structure and properties as well as particle shape and therefore is not a suitable method when characterization of the pristine protein is desired.

For this work, protein formulation was spiked with degraded polysorbate to induce protein particle formation. The sample was then placed into a liquid cell. Submicron particles were counted; their size and morphology determined, followed by Raman spectroscopy analysis.

The results are summarized in the Figure shown below. An area of the liquid cell was analyzed and a particle size distribution table was generated. In a fully automated process the instrument chose a selection of particulates, aligned and exposed them to the Raman laser. The spectra were then processed and compared with the spectral library. As indicated in the table, many protein particles were detected in the sample.

The use of the liquid cell enables characterization of pristine protein particles and helps to avoid sample preparation that can introduce undesired changes to the samples. Furthermore, it helps to maintain the shape of proteinaceous particles that can be altered or lost during filtration. This helps to correlate shape, morphological information and chemical composition acquired through Raman spectroscopy. The combination of this data can provide valuable information on the pathways of particle formation as well as formulation stability data.

Keywords: Instrumentation, Microspectroscopy, Pharmaceutical, Protein
Application Code: Pharmaceutical
Methodology Code: Molecular Spectroscopy
The uniformity of active pharmaceutical ingredients (APIs) within the tablet can affect drug bioavailability and tablet stability. Rapid analysis techniques designed to qualify and quantify APIs in tablet and powdered mixture are needed in various pharmaceutical applications, including stability assessments and formulation process monitoring. Second Harmonic Generation (SHG) microscopy is a great tool for sensitive (ppm level) and selective detection of chiral crystals. Due to its own inherent capability of detecting chiral crystals, SHG microscopy is a potential imaging modality for rapid (few milliseconds to map out 500x500 μm² area of interest) selective identification and quantitation of non-centrosymmetric crystals of APIs during the formulation process. However, the presence of multiple SHG active crystalline materials may complicate the quantitative measurements of APIs. On the other hand, Raman imaging, which is highly specific and chemical information rich technique, is widely used to acquire chemical information qualitatively and quantitatively. But the significant amount of time consumption limits the high throughput screening during the formulation process. An instrument combined SHG microscopy and Raman spectroscopy has been developed for rapid qualitative and quantitative measurements of APIs. High throughput and rapid Raman measurements were achieved from a reduced number of sampling based on SHG image. In order to facilitate the random access Raman spectroscopy, conventional resonant-galvo scan head has been replaced with a galvo-galvo scan head to send the laser beam at the exact position of interest. SHG guided Raman spectroscopy has been demonstrated for rapid chemical identification and quantification of powdered mixture of a model API (griseofulvin) and common pharmaceutical excipients within a few seconds. The discrimination and quantification of different polymorphs of APIs was also achieved.
Elemental impurities in pharmaceutical products may arise from different sources. There has been much interest in the presence of toxic elements in pharmaceutical products and excipients recently. New guidelines regarding elemental impurities in pharmaceutical products will be implemented in January 2018. In this work a method for analysing arsenic (As), cadmium (Cd), mercury (Hg), lead (Pb), cobalt (Co), nickel (Ni) and vanadium (V) was developed and validated to meet the international conference of harmonisation (ICH Q3D) guidelines using inductively coupled plasma optical emission spectroscopy (ICP-OES) and inductively coupled plasma mass spectrometry spectroscopy (ICP-MS). Sample preparation was performed using microwave assisted acid digestion (CEM SP-D) method was developed using reverse aqua regia and for tablets grinding was found to be necessary to minimise variation before digestion. The obtained results from the validation showed good linearity ($R^2>0.995$) over a wide range with low limits of detections (LODs) and limits of quantification (LOQs). The percentage recoveries for both the standard reference material (SRM) and the spiked samples were between 95-105% with relative standard deviation (RSD) of less than 5. Commercially available analgesics and cough remedies were purchased and analysed. Six out of the eight products have Pb levels exceeding the permitted daily exposure limit (PDE) when the maximum dose is taken and four products contain Cd in concentration that exceeds the ICH Q3D PDE. Other elements were also quantified in some of products including Hg, Co and Ni, but were within the PDEs. A comparison of the two techniques will be carried out taking into consideration the selectivity and sensitivity of the method.

The following LODs and LOQs (ng/ml) were obtained using ICP-OES:

- As: 1.95, 5.86
- Cd: 0.29, 0.87
- Hg: 0.8, 2.3
- Pb: 1.56, 4.73
- Co: 0.77, 1.58
- Ni: 0.74, 1.72
- V: 2.75, 7.64

Keywords: ICP, ICP-MS, Metals, Pharmaceutical

Application Code: Pharmaceutical

Methodology Code: Atomic Spectroscopy/Elemental Analysis
The introduction of the new ICH Q3D regulations for Elemental Impurities (EI) in pharmaceutical formulations is already in effect for new chemical entities (NCE) and is coming into effect for all formulations on the 1st January 2018. This has caused an urgent requirement for validated methodologies to be made available.

This work will consider some of the issues with the new regulation with specific reference to As analysis in flu remedies as well as antimony in the treatment of Leishmaniasis. The work will compare three techniques ICP-OES, ICP-MS and hydride generation atomic absorption spectroscopy.

Optimisation of a microwave digestion technique will be discussed using a CEM SP-D digestion system. In each technique this was shown to obtain recoveries of 95-105% for As. The digestion methods were validated using NIST 3280 Multi-mineral/Multi-vitamin standard. This SRM has limitation when compared to the regulation requirements such as the concentration of the elemental impurities in the SRM which are ten fold lower than the regulations. The SRM has limitations to the range of elemental impurities with Hg not present in the standard. The optimum temperature for digestion was found to be 210 C. The NIST 3280 SRM will be discussed with reference to a new material produced for an IPEC round robin study of elemental impurities.

For each technique optimisation of the method using signal and signal/blank ratio will be discussed. Each technique will be compared with the requirements ICH Q3D regulations.

In the case of flu remedies it was clearly demonstrated that As was present in each of the formulations tested. The levels were between 30-40% of the PDE for As in oral formulations. This was as a direct result of the large mass of the maximum dose and the requirement for intake not to exceed 15 {micro}g/day.

Keywords: Hydride, ICP, ICP-MS, Pharmaceutical
Application Code: Pharmaceutical
Methodology Code: Atomic Spectroscopy/Elemental Analysis
Session Title: Pharmaceutical Characterization with Spectroscopy and Spectrometry
Abstract Title: Testing and Validation of Various Antacids – Trace Elemental Impurities and Major Components in a Single Analysis

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Abstract Text:
With the implementation of USP chapter 232 and ICH Q3D to marketed products, some medications will have a requirement to routinely test for trace levels of toxic metals using ICP-MS. In some cases the active ingredient will also contain a species which is amenable to analysis by atomic spectroscopy techniques such as Calcium, Magnesium or Aluminum. Typically these elements are currently measured in quality control tests using titrations or Atomic Absorption which require lengthy sample preparation procedures and have low sensitivity towards impurities. We demonstrate a validated methodology for simultaneously quantitating the high and low level elemental species using ICP-MS.

Keywords: Elemental Analysis, ICP-MS, Pharmaceutical, Trace Analysis
Application Code: Pharmaceutical
Methodology Code: Atomic Spectroscopy/Elemental Analysis
Pharmaceutical Characterization with Spectroscopy and Spectrometry

Development of an Instrument for Rapid Characterization of Crystal Content in Pharmaceuticals Using Triboluminescence

Triboluminescence enables rapid detection of residual trace crystallinity within nominally amorphous drug formulations by taking advantage of the piezoelectric properties of most chiral crystals. Many active pharmaceutical ingredients (APIs) in use today use an amorphous form of the active ingredient due to the higher bioavailability compared to its more thermodynamically stable crystalline form. In optimizing amorphous solid dispersions, the desire to reduce the drug loading to minimize the probability of crystal formation during storage is countered by the desire to maximize drug loading to reduce the overall size of the final dosage form. These competing desires typically lead to extensive screening of polymers and polymer blends. However, current routine methods of detecting crystallinity in a compound generally exhibit detection limits on the order of a few percent, which are comparable in some instances to the total drug loading. In this talk, we present a simple instrument compatible with the needs of process monitoring that is capable of rapidly and selectively detecting crystal content in solid dispersions of homochiral APIs. The instrument relies on the phenomenon of triboluminescence, or the emission of light upon mechanical disruption. Except for a few relatively rare high symmetry cases, chiral crystals are symmetry-allowed for piezoelectricity, or the generation of electric fields upon distortion of the lattice, which in turn contributes to triboluminescence. By physically stressing a sample we are able to detect small amounts of crystals by detecting photons emitted from the triboluminescence of fracturing crystals with detection limits well below 1%.

Abstract Text

Keywords: Instrumentation, Pharmaceutical, Sample Handling/Automation

Application Code: Pharmaceutical

Methodology Code: Process Analytical Techniques
The first two grinding (break) operations of the milling process that open individual wheat kernels are essential to release the maximum amount of pure endosperm from the kernel. At early stages of the operation, extraction of enough wheat endosperm is essential for further processing with the expressed intent of maximum production of flour. The third break operation effectively controls the maximization of the overall straight grade flour yield. The product efficiency at this stage can make or break the cost/benefit ratio of the energy necessary to recover the remaining product from the wheat bran. Quantitative chemical imaging is an ideal technique to analyze wheat flour (product) from early stages of the product isolation process. The technique is not specific to the location of the endosperm within the wheat kernel. This is advantageous because much of the remaining endosperm at this stage is adhered closely to the bran. Examples are presented for third break streams and various subsequent streams to indicate the distribution of endosperm in this important milling process.
As a process analytical technology, Raman spectroscopy is a powerful tool that provides real-time in situ measurements that inform on the process chemistry. Representative sampling of the process 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, which affects the sampling volume of an optical probe and recovery of Raman-scattered photons. New approaches in sampling for Raman spectroscopy provide representative sampling in solids and turbid media and enable in-process corrections. 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. In a representative example we discuss how a hybrid approach using backscattered Raman and wide area Raman can provide multi-scale measurements in-process in order to improve understanding of a heterogeneous polymerization reaction. Similar examples in pharmaceutical and bioprocessing will illustrate Raman’s capability for providing robust measurements in process.

Keywords: Biopharmaceutical, Pharmaceutical, Polymers & Plastics, Raman Spectroscopy
Application Code: Process Analytical Chemistry
Methodology Code: Vibrational Spectroscopy
A Novel FTIR-GC/FTIR Detection Method as Applied to Process Monitoring of Carbon Dioxide Purity

Carbon dioxide sees many uses in industrial processes, from beverage carbonation to semiconductor fabrication and pharmaceutical production. These processes require the stringent monitoring of possible impurities in the carbon dioxide bulk which could result in poor quality taste of a beverage or contamination of a silicon wafer or a drug formulation. The most common techniques used for purity monitoring involve GC/MS, fluorescence or NDIR. We have developed a technique which couples the separation power of GC with the spectroscopic resolving power of FTIR in order to both accurately speciate and quantitate impurities in carbon dioxide with the added capability of sample pre-concentration using thermal desorption tubes. In addition, the analyzer can be used in a stand-alone FTIR mode to analyze for materials at much higher (ppmv to % level) concentrations. Analyses can be performed in a matter of 10-30 minutes, making this a rapid and accurate means for detection of impurities designed for process monitoring. Test data in which prescribed amounts of impurities are introduced into 99.999% CO2 will be discussed.

Keywords: FTIR, GC
Application Code: Process Analytical Chemistry
Methodology Code: Process Analytical Techniques
A Novel Approach for Accurate On-Line Capillary Column Heating Using Micro-Convection Oven Technology

The implementation of high resolution capillary gas chromatography on-line has been difficult due to the lack of availability of a highly accurate heated column oven. For the sake of safety, most process gas chromatographs utilize forced air heating for their primary oven control. This technique works well for packed column chromatography (where the column’s material provides heat capacity); but fails to deliver the very stable retention time repeatability required by a modern fused silica capillary due to the technique’s inherent thermal hysteresis. This problem is further complexed by the aggressive oven ramping techniques desired for fast chromatography techniques.

While others have tried to resolve this problem using alternative technologies, most of these require special preparation of the capillary column. The Wasson-ECE R&D team has been working to address the basic design limitations that have prevented the implementation of convection column ovens in the process environment. This presentation will highlight a new convection oven, specifically designed for implementation in process gas chromatographs. The use of modern insulation materials and novel oven geometries have resulted in an oven that matches the performance found in modern laboratory GCs. Retention time stability and oven performance data will be presented.

This new oven technology will allow for the implementation of hyphenated techniques that have not been commonly used on-line (GC-VUV, GC-MS, GC-ICPMS, GCxGC, etc.). Overview of the application of these technologies on-line will be presented.

Keywords: Capillary GC, Gas Chromatography, Gas Chromatography/Mass Spectrometry, GC-MS
Application Code: Process Analytical Chemistry
Methodology Code: Process Analytical Techniques
Instrumentation was developed for rapidly assessing the qualitative presence of crystallinity within pharmaceutical powders and slurries based on impact driven triboluminescence (TL). The solid state form of an active pharmaceutical ingredient (API) can profoundly impact its efficacy. In amorphous formulations, even trace crystallinity can potentially reduce the shelf life by providing nucleation sites, which can lead to a decrease in bioavailability upon crystal formation. Two complementary platforms for TL detection are demonstrated. Focused ultrasonication is used for generating TL signal from slurries in a suspension, and solenoid-driven impaction is used for powder samples. The instrument was tested using both griseofulvin crystals mixed with polyethylene glycol (PEG) and sucrose crystals. The TL signal was shown to have a particle size dependence, a linear relationship to percent crystallinity, and a limit of detection of 0.3%. Measurement times for powder samples are ~1 s and ~1 ms for slurry samples. The low detection limit, high throughput measurement, and simple yet robust construction are compatible with the demands of process analytical technology.

The NSF-CHE/CMI program and Trask innovation fund from Purdue University are acknowledged for funding this work.
Holographic video microscopy can detect individual micrometer-scale protein aggregates and measure their size and refractive index [1]. The measurement proceeds fast enough to build up population averages for time-resolved studies and lends itself to tracking trends in protein aggregation arising from changing environmental factors. We demonstrate these capabilities through measurements of protein aggregates in a variety of protein samples including bovine serum albumin, human IgG, and oxytocin. The protein aggregates were characterized as a function of buffer ionic strength, pH, temperature, and mechanical stress through sonication. By detecting individual aggregates, we found that higher pH leads to increased aggregation in oxytocin solutions. In addition, our results demonstrate that holographic characterization can detect differences in protein aggregate distributions by changes in refractive index. After thermal cycling and sonication, the refractive index of human IgG protein aggregates showed significant differences from their previous distribution before modification. Finally, we also measured protein samples in the presence of additives used to minimize protein aggregation. Holographic characterization measurements are compared with results obtained with other particle characterization methods.


Keywords: Biopharmaceutical, Particle Size and Distribution, Process Monitoring, Protein
Application Code: Pharmaceutical
Methodology Code: Process Analytical Techniques
**Abstract Text**

Sexual assault samples sent to forensic labs are often examined for the presence of male DNA from sperm cells. In order to reduce cost, only samples containing male DNA are then further processed for STR analysis and uploaded to a database. The screening process can be achieved via microscopy or by PCR using a male-specific marker. These processes can be subjective, costly, and laborious. The method we developed here utilizes a unique enzymatic DNA liberation step followed by loop mediated isothermal amplification (LAMP) targeting a male marker. The reaction is then assessed by a color change via cell phone imaging, providing a digital readout to identify samples containing male DNA. LAMP has immense potential for rapid screening of samples for several reasons: (1) amplification is done at one temperature compared to thermocycling in standard PCR, reducing instrumentation complexity, (2) the colorimetric detection by smartphones negates the need of complex hardware normally associated with fluorescence detection, (3) four primer pairs designed for LAMP ensure high specificity and minimize false positive reactions, (4) detection limit for LAMP is as low as 25 pg, which allows minimal sample consumption and suggests as few as 9 sperm cells can be detected, (5) LAMP is compatible with upstream processes common to forensic laboratories and could be readily multiplexed onto a 96-well plate. The method provides a simple yet reliable screening procedure for male DNA in under 1 hour from sample preparation to analysis. It has great adoptability in forensic laboratories, can be automated, and has potential for point-of-analysis applications.

**Keywords:** Bioanalytical, Biological Samples, Forensics, Method Development

**Application Code:** Bioanalytical

**Methodology Code:** Process Analytical Techniques
Enzymes are difficult to purify and obtain in large quantities. Hence, it would be advantageous to develop bioanalytical methodologies that are cost-effective and provide efficient multiple uses of enzyme catalysts for biocatalytic and biosensing applications. We present here biocatalytic and electrocatalytic properties of purified and membrane-bound enzymes incorporating magnetic nanoparticles (MNPs). We demonstrate that by appropriate selection of functionalized MNPs to immobilize purified or membrane-bound forms of enzymes, one can achieve cost-effective, stable, scalable, and reusable biocatalytic systems for green synthesis of fine chemicals, specialty materials, and biosensing applications. The advantages of protein immobilization with MNPs include convenient preparation, efficient free protein recovery, effective reusability, easy product isolation, and long-term storage stability of protein-bound MNPs in comparison to the free protein solutions.

Keywords: Bioanalytical, Biopharmaceutical, Biosensors, Electrode Surfaces

Application Code: Bioanalytical

Methodology Code: Process Analytical Techniques
Small variations in the manufacturing processes of plastics, adhesives and sealants can be detrimental to the dimensional stability of the product. Current screening methods quantify outgassed materials by total mass loss (TML) but do not accurately identify compounds in the sample matrix. By determining the outgassing rate for individual species, manufacturers of adhesives can better assess contamination and control for process deviation, eliminating costly production errors.

This paper provides a novel approach to both speciating and quantifying outgassed materials from adhesives using a new analytical technology that combines the separation of gas chromatography with the quantitative analysis of absorption spectroscopy. An adhesive sample was sealed in a closed-loop circulating system through an FTIR gas cell for continuous monitoring of outgassed materials. Since each component has a unique IR spectrum, this method allows for identification of the emitted solvents and calculation of their individual outgassing rates.

To further characterize the sample matrix and detect even lower level emissions, thermal desorption tubes (TDTs) were sampled and analyzed using GC-FTIR. The aggregate results of these two techniques provide outgassing rates of major solvents as well as a full profile of outgassed materials, detectable down to ppb (ng) levels.

Keywords: FTIR, GC, Process Monitoring, Trace Analysis
Application Code: Process Analytical Chemistry
Methodology Code: Process Analytical Techniques
Frequently, the analytical tools used for process stream characterization will provide a slower data rate than desired. The results may represent only periodic snapshots from a changing process stream and as a result process variation may be hidden from observation. More rapid results may be possible but often the results represent trends that still present a fuzzy observation of the actual process conditions.

Mass Spectrometry (MS) can be used for direct process gas measurements. MS can be performed rapidly in a real-time manner to allow characterization of many process stream variations. However, MS techniques must be connected directly to a process stream with caution. Large operating pressure differences, MS sensitivity and unexpected high concentrations in the process stream represent a few of the challenges for direct MS measurements. These challenges along with design strategies will be discussed in this presentation. A Real-time Gas Analyzer (RTGA) using Mass Spec (MS) detection (RTGA-MS) has been developed to provide a reliable, sensitive, real-time chemical measurement tool for continuous process improvements. There is no GC required for this type of real-time analysis, making this an ideal technique where very fast analysis is required, such as monitoring transients and continuous reactions. Applications include fuel cell gas analysis, syngas monitoring, reactor monitoring and others. Quantitation is quite easy and reproducible for most gases, including hydrogen, using a readily available benchtop single quadrupole mass spectrometer detector (MSD).

**Keywords:** Analysis, Detection, Process Monitoring, Quantitative

**Application Code:** Process Analytical Chemistry

**Methodology Code:** Process Analytical Techniques
Several model microorganisms are currently used in the biological field to understand more complex phenomenon. Among these microorganisms, fungus are widely used because of some special features such as velocity of grown, simplicity of manipulation and possibility of genetic medication. In this context, the present work shows results of in vivo studies on degradation of hydrogen peroxide (H$_2$O$_2$) in Aspergillus fumigatus fungus. The experiments were carried out inserting a platinum microelectrode (r = 3 µm) inside a wild fungus immersed in liquid solution (culture medium). In order to perform local measurements, the external body of the microelectrode was covered by silver glue to get a dual function electrode, where the silver coat acted as a pseudo reference electrode. Cycling voltammograms were performed sequentially before and after addition of a H$_2$O$_2$ solution in the culture medium. Fig. 1 shows a typical microelectrode response as a function of time. Current was almost constant before the addition of H$_2$O$_2$ (basal signal), whereas a significant increase was noticed after addition of H$_2$O$_2$ as a consequence of its diffusion from the culture medium to inside the fungi. The current decrease observed after the addition of H$_2$O$_2$ is related to the presence of the peroxiredoxin protein, which can degrade H$_2$O$_2$ inside the microorganism.

Keywords: Bioanalytical, Biological Samples, Detection, Electrode Surfaces

Application Code: Bioanalytical

Methodology Code: Electrochemistry
Surface tethering of peptides, proteins, and the like creates functionalized surfaces with excellent properties. Such methods of immobilization often produce surfaces with less than desired results often due to the lack of control in how such molecules are covalently (or even physically) bound to the surface. This work demonstrates an application of site-specific mutations on beta-galactosidase that allow for chemical immobilization with well-defined orientations. Furthermore, a combination of sum frequency generation (SFG) vibrational spectroscopy and coarse grain molecular dynamics simulations were used as tools to determine effect of immobilization site and surface chemistry and how these factors influence enzyme surface coverage and overall catalytic activity. Results suggest that for two mutations in close proximity (E227C and D308C), orientation, surface coverage, and catalytic activity are measured to be nearly identical. When the tethering surface was made more hydrophilic, by the addition of hydroxide, surface coverage decreased but activity increased. This work helps to guide in the understanding of how enzyme tethering can be optimized using several parameters to maximize catalytic activity.

Keywords: Characterization, Protein, Surface Analysis, Vibrational Spectroscopy
Application Code: Bioanalytical
Methodology Code: Vibrational Spectroscopy
Ammonia is an important compound to many industries around the world. Currently, the production of ammonia is by the Haber-Bosch process, which requires large amounts of energy as well as high temperatures and pressures.

In this report, an alternative method for ammonia production is explored. With [i]Anabaena Variabilis[/i], a photosynthetic cyanobacteria, on a carbon electrode, ammonia can be generated at ambient temperatures and pressures at little energy cost, a few tenths of a volt [1, 2]. A bioelectrocatalytic device has been constructed by immobilizing whole cell [i]a. variabilis[/i] in a Nafion film modified with a trimethyl octadecyl ammonium bromide (TMODA) salt at an electrode surface [3]. The polymer modified electrode provides the driving force and reductive microenvironment to facilitate production of NH[sub]3[/sub] by nitrogenase in heterocystic cells and nitrate/nitrite reductase in vegetative cells present in [i]a. variabilis[/i]. Ammonia production by cyanobacteria were increased from basal levels of 2.8 ± 0.4 µM produced over a two week period, to 22 ± 8 µM produced in 20 minutes under mild voltage perturbation, roughly 104 increase in rate. Chronoamperometric perturbation studies show increased ammonia production at near +600 mV and -300 mV vs SCE. In cyclic voltammetric studies, nitrate/nitrite reductase in vegetative-only biofilms responds favorably to positive voltage ranges, while isolated heterocyst biofilms containing nitrogenase can be effectively targeted with the application of a negative voltage profile.

References:

Keywords: Bioanalytical, Electrochemistry, Potentiometry, Voltammetry
Bioanalytical - Electrochemistry

Experimental Evaluation of Titanium Substrate Photofunctionalization Effect on Proliferation and Cell Differentiation of Fibroblasts

This study indicates the influence of Ti surface state on cellular proliferation/differentiation/activation. It is well known that the photofunctionalization by ultraviolet (UV) irradiation to titanium (Ti) substrate markedly promotes the osteogenesis. Photofunctionalization is surface processing technique for improving implant surfaces such as orthopedic/prosthetic-implant by luminous rays having specific wavelength and designated strength. The adhesiveness and function expression of cells are considered to influence by the surface state (hydrophilic, hydrophobic, roughness, etc.) of substrate materials. It is common knowledge that cells strongly adhere to a UV irradiated Ti substrate because affinity of Ti surface and cells is improved by UV surface modification. However, details of these phenomenon and mechanisms are not clarify.

In this study, the influence of surface modification of Ti substrates to cells was examined based on method of physical chemistry and biochemistry. The pure Ti substrates (surface roughness is 0.1 μm and 1 μm) were used as experimental samples. The excimer lamp and low-pressure mercury lamp were used for photofunctionalization process of Ti substrates. Also, ozone treatment of Ti substrate was carried out for comparison examination. The osteoblast cells (MC3T3) and the myoblast cells (C2C12) were used for evaluating the photofunctionalization effect of the Ti substrate. The adhesion/proliferation/differentiation state of cells on the Ti substrate that was processed by the various UV irradiation conditions were evaluated using fluorescence microscopic observation and gene expression analysis. We would like to discuss the relationship between the physicochemical state of Ti surface and proliferation, differentiation, and activation of cultured cells.

Keywords: Bioanalytical, Genetic Engineering, Immunoassay, Surface Analysis
Application Code: Bioanalytical
Methodology Code: Biospectroscopy
Protonation of Fe(OEP)NO was studied in the presence of substitute phenols using chemical and electrochemical reduction of Fe(OEP)NO. When the UV-visible spectrum of reduced Fe(OEP)NO was obtained using spectroelectrochemistry in the presence of phenols, the bands related to Fe(OEP)NO disappeared and new bands similar but not identical to Fe(OEP)NO were obtained. In the presence of phenolate, the spectrum of Fe(OEP)NO was seen. In order to further investigate the protonated species, the proton NMR spectrum was taken with chemically reduced Fe(OEP)NO and Fe(OEP)[15]NO in the presence of phenols. An acidic proton was observed at the chemical shift 12.60 ppm for HNO. The peak disappeared in the presence of the phenolate base. Fe(OEP-d[4]NO) was synthesized to confirm that the resonance did not come from meso protons of the porphyrin macrocycle. A NOE experiment showed that an exchange was occurring between the hydrogen of HNO and phenol. The resonance due to HNO was strongly temperature dependent. The kinetics of the protonation on Fe(OEP)NO were studied by electrochemical methods. The reaction was reversible but the re-oxidation of Fe(OEP)NO was slow and limited by deprotonation. This phenomenon was confirmed by UV-Visible spectra. The FTIR spectroelectrochemistry of Fe(OEP)NO in the presence of phenols was also carried out.

Keywords: FTIR, NMR, UV-VIS Absorbance/Luminescence, Voltammetry

Application Code: Bioanalytical
Methodology Code: Electrochemistry
Abstract Text

Biological fluids are highly complex samples that contain an abundance of medically relevant information. Being able to detect and identify low level metabolites in these samples without pretreatment or expensive equipment would be an attractive diagnostic tool. Surface-enhanced Raman scattering (SERS) is a sensitive spectroscopy technique that allows for label-free molecular specific identification. Our lab uses sheath flow SERS to concentrate analytes onto a silver nanostructure substrate where the SERS signal will be generated. The combination of capillary electrophoresis and sheath flow SERS enables high resolution separation and detection of low level metabolites in biological samples and fluids without pretreatment. Previously, our lab detected low level biological species, such as biologically-active peptides, using a custom capillary electrophoresis sheath flow SERS set-up. We have extended the capabilities of this instrument for the separation of untreated and unlabeled metabolites in urine and serum. Our work suggests that capillary electrophoresis coupled with SERS detection could be a novel platform for biomedical diagnostics.

Keywords: Capillary Electrophoresis, Surface Enhanced Raman Spectroscopy
Application Code: Bioanalytical
Methodology Code: Vibrational Spectroscopy
Chemotherapy-induced cognitive impairment, also known as ‘chemobrain’, is a medical complication of cancer treatment that is estimated to affect one-third of patients who receive chemotherapy treatment. Chemobrain is characterized by a general decline in cognition affecting memory, attention, motor function, and complex problem-solving skills. Alterations in the release and uptake of dopamine, a neurotransmitter that plays a crucial role in cognition, could potentially contribute to chemobrain. To investigate this, fast-scan cyclic voltammetry (FSCV) at carbon-fiber microelectrodes was used to make in vivo measurements of dopamine and its metabolites in zebrafish. Zebrafish, a small subtropical fish, have become a popular animal model in neuroscience due to several advantages they have over rat models, such as high throughput, ease of treatment, and large offspring production. Our previous study has shown that carboplatin, a chemotherapeutic drug, alters dopamine release and affects cognition in rats, especially in spatial learning discrimination. Here, for the first time, we employed zebrafish as a chemobrain model, treating wild type zebrafish with 5-fluorouracil (5-FU) for one, four, and seven days. 5-FU was administrated by feeding mature fish with pre-treated brine shrimp (20 mg drug / kg food). The whole brain was harvested at each treatment period and electrically evoked dopamine release was compared to control groups. 5-FU treated zebrafish exhibited a statistically significant decrease in dopamine release compared to control zebrafish at each treatment period, suggesting that 5-FU contributes to chemobrain by inhibiting neurotransmitter release.
Nickel based material is one of the promising electrodes used in electrochemical sensor for sensing of small organic molecules due to its high conductivity and good electrocatalytic property. To increase the electrode performance, a combination between nickel and other nanometerials (i.e. metal xide, carbon based material) has become more attractive for electrode surface modification. Herein, a new combination of nickel phosphorus – titanium dioxide – reduce graphene oxide (NiP-TiO2-RGO) modified electrode surface was successfully prepared by using one-pot electroless deposition and used for the determination of small organic molecules (i.e. glucose). In this study, the parameters controlling the surface morphology and the electrochemical sensitivity of NiP-TiO2-RGO electrode, such as amount of TiO2 sol, amount of GO and electroless deposition time were investigated and optimized. Then, the surface morphology of Ni-P-TiO2-RGO electrode was characterized by scanning electron microscopy (SEM) and transmission electron microscopy (TEM). The electrochemical sensitivity of this electrode was invetigated by cyclic voltammetry. The TEM and SEM images verify the well dispersion of NiP-TiO2-RGO solution and the homogenous coated electrode surface leading to increased sensitivity and improved reproducibility of the sensor. Finally, this system was successfully applied for a non-enzymatic detection of glucose. All of these results will be presented.
Glucose oxidase (GOx) is an enzyme, which is the most frequently applied in the construction of electrochemical biosensors [1]. Electron transfer mediators (ETM) are mostly applied in order to facilitate the ET from GOx towards electrode, because the redox centre of GOx, which in redox centre possess the flavin adenine dinucleotide (FAD), which is deeply embedded within GOx molecule and during the redox processes that are catalysed by GOx the electrons, could be not directly passed to the electrode surface. ETMs, which have a lower oxidation potentials than the redox centre of GOx, can be used for electron transfer from glucose oxidase to electrode. Therefore the electron transfer from the enzyme to selected redox mediator must also occur rapidly to overcome the kinetic barrier.

In this research some aspects of electron transfer (ET) in electrochemical system based on immobilized gold nanoparticles (AuNPs) and glucose oxidase (GOx) were investigated. It was shown that in the design of AuNPs and GOx modified graphite rod (GOx/AuNPs/GR) electrode the AuNPs are facilitating the electron transfer from GOx towards graphite rod electrode. It was determined that significant electrode currents and well-defined oxidation/reduction peaks were registered at potential sweep rate of 0.1 V s-1. Anodic peaks in cyclic voltammograms, which were observed in the presence or absence of glucose (Glu), were proportional to the square root of the potential sweep rate (v1/2) in the range of 0.045-0.316 (V s-1)1/2, which is indicating that the electrochemical reaction is diffusion controlled.

Bioanalytical - Electrochemistry

Adenosine Monophosphate Capped Graphene Quantum Dots for Selective Detection of Dopamine

Dopamine plays a critical role in brain health. Sensitive detection of dopamine in biological samples is in high demands. In this work, we developed a graphene quantum dots (GQDs) based on fluorescence sensor for the detection of dopamine. GQDs have been widely used in vitro or vivo imaging due to its low toxicity and good biocompatibility. In addition, graphene quantum dots have oxygen-containing functional groups for further modification. In this study, we designed a high fluorescence graphene quantum dots capped with adenosine monophosphate. Dopamine molecules were positively charged (pH=7.4) while adenosine monophosphates were negatively charged. With electrostatic interaction, dopamine will naturally bind to it via non-covalent binding. The alteration of the surface environment for quantum dots will influence the optical properties and eventually quench the fluorescence of the quantum dots owing to the recombination of the electron hole pair. The decreased fluorescence intensity is proportional to the concentration of dopamine in biological samples.

Keywords: Bioanalytical, Biosensors, Detection, UV-VIS Absorbance/Luminescence

Application Code: Bioanalytical

Methodology Code: Fluorescence/Luminescence
Copper nanoparticles (CuNPs) formed on the DNA scaffold have showed outstanding functionality in analytical biosensors as fluorescence probes. To overcome the limitation of monomeric CuNPs, improving their fluorescence intensity, a signal amplification strategy by using hybridization chain reaction (HCR) technique was introduced into the system. Two hairpin structure probes were designed not to initiate HCR in their free state but activated HCR on binding to the target oligonucleotide. After adding of copper ions and ascorbate, the formed CuNPs using dsDNA template with repetitive units could emit high intensive fluorescence at 596 nm under a 340 nm excitation. The fluorescence intensity of CuNPs was around 5000 folds enhanced after and before HCR. The microRNA mir-21 based method showed excellent sensitivity (with detection limit of 100 pM) and high selectivity. This method also might be widely applied to other oligonucleotides analytes, even non-oligo analytes after ingenious design.
Immobilization of single strands of DNA on a solid surface and subsequent hybridization with the complementary sequence are the foundation of many devices such as DNA biosensors, nanostructures, gene chips, DNA computers and therapeutic devices. A considerable number of reports employing surface hybridization reactions, in which sequence-specific recognition occurs between immobilized and solution nucleic acids are presented in the literature, although hybridization is not completely understood in bulk solution. The present work aims to provide a fundamental hybridization/electrochemical understanding of redox-DNA strands by using a methylene blue labelled probe DNA modified gold electrode and square wave voltammetry as the excitation/measurement technique. After modification of the gold surface, the methylene blue at probe DNA distal end is in close position to the underlying electrode in such way that electrons can be transferred easily. Upon hybridization with the target ferrocene labelled DNA a more rigid duplex is obtained decreasing the apparent rate of electron transfer between methylene blue and gold electrode. Additionally, as the target DNA contains a ferrocene label at its distal end, a second electrochemical process will occur related to the ferrocene redox reaction. An unusual regime is observed in which small faradaic currents for the ferrocene labelled target DNA are obtained, while the signal suppression for the probe label signal is obtained as expected. A method for the calculation of the surface coverage and rate constant is provided, which can be used for monitoring the DNA.
Pelvic organ prolapse (POP) is a debilitating condition characterized by weakening of pelvic floor connective tissues and loss of support for pelvic organs. Collagen is the predominant structural protein within the tissues. Our previous study has shown that the ratio of collagen I (COLI) and collagen III (COLIII) is 1.4 in normal tissues, but is doubled in the tissue of POP patients with a concomitant reduction of the amount of total collagen leading to loose and fragile fiber network accountable for the weak load-bearing capability. The aberrant collagen expression in POP tissue is likely due to the abnormal synthesis and degradation of collagen by fibroblasts. In this work, we extracted fibroblasts from tissues and manipulated their culture matrix properties, e.g., biochemical composition, fibrous alignment and mechanical strength, to boost the total collagen synthesis and amend the COLI/COLIII ratio. The results show that aligned COLI/COLIII fiber matrices, mimicking the composition of collagen in connective tissues, effectively activated fibroblasts for collagen synthesis. However, the produced collagen has a COLI/COLIII ratio of 2.8:1, close to that in POP tissues. While the addition of CNT to collagen further stimulated fibroblasts, a silk/CNT fiber matrix significantly elevated the COLIII production to reach a COLI/COLIII ratio of 3:5 in addition to the increase of total collagen synthesis by two folds. The capability of stimulating cells through the engineering of biopolymers offers the opportunity of developing personalized cell therapy for noninvasive treatments. It also inspires the design of effective prosthetic materials for use in prolapse surgery.
Laminated Microfluidic Paper-Based Analytical Devices for Clinical Protein Assays

Microfluidic paper-based analytical devices ([micro]PADs) have gained a lot of attention for their attractive features owing to the use of paper as a substrate, such as being low-cost, easily and safely disposable by incineration and allowing pump-free sample transport driven by capillary forces. However, it is still challenging to perform sub-microliter sample analyses by [micro]PADs, mainly because of large dimensions of microfluidic structures and the open system prone to evaporation of the sample liquid. In this work, we demonstrate the advantages of using a hot laminator instead of a hot plate in the wax printing-based microfluidic patterning method. The shortened heating time and the pressure applied to the paper substrate by the hot rollers contribute to the formation of high-resolution microfluidic structures. Consideration of the device geometry and the influence of cellulose fiber direction in the filter paper substrate have led to a model [micro]PAD design with four microfluidic channels that can be filled with as low as 0.5 [micro]L of liquid. A colorimetric protein assay was performed targeting tear fluid protein analysis. This [micro]PAD allows to obtain quadruplicate colorimetric data by single pipetting of a sub-microliter sample. Finally, the strength of fully enclosed microfluidic structures in [micro]PADs, achieved by device lamination, is demonstrated for sample volume-independent quantitative assays. Prevention of evaporation by lamination leads to controlled sample liquid uptake, resulting in constant colorimetric signals regardless of the sample volume applied to the [micro]PAD, which is a significant advantage for practical applications.

Keywords: Analysis, Clinical Chemistry, Lab-on-a-Chip/Microfluidics, Paper/Pulp
Application Code: Biomedical
Methodology Code: Microfluidics/Lab-on-a-Chip
Biomedical Analysis

Miniature Gas Chromatography Based Breath Analyzer for Non-Invasive Point-of-Care Diagnostics of Acute Lung Injury

The recognition, diagnosis and management of acute lung injury from infection or trauma is very challenging due to its dependence on century old use of radiographs, bacterial cultures and other crude techniques. In recent years there is increasing research on breath analysis, which describes the relation between volatile organic/inorganic compounds (VOC/VIC) biomarkers and lung diseases like lung cancer, chronic obstructive pulmonary disease (COPD) and acute respiratory distress syndrome (ARDS). The non-invasive nature of breath analysis makes it suitable for early diagnosis of respiratory diseases.

Due to the complex composition of exhaled breath, gas chromatography coupled with mass spectrometry (GC-MS) is the most established method for breath analysis. However, clinical implementation of GC-MS is quite challenging because of its low mobility, high cost and requirement of experienced personnel.

We have developed a rapid, sensitive and fully automated breath analyzer based on miniature gas chromatography to discriminate patients with acute lung injury and healthy controls. The breath analyzer collects the exhaled breath from a mechanical ventilator for 15 mins from intubated patients. The collected breath undergoes analysis for another 15 mins to identify and quantify target VOC/VIC biomarkers. The VOCs in the breath are pre-concentrated in a pre-concentrator that is packed with Carboxen\textsuperscript{TM}-1000 and Carbopack\textsuperscript{TM}-B adsorbents during sampling, then injected to Rtx-1 column for separation, and detected by 10.6 eV photoionization detector (PID) at the end of the Rtx-1 column. The VICs are stored in a sampling loop and then injected to porous layer open tubular (PLOT) column for separation and detected by helium discharged photoionization detector (HDPID) at the end of the PLOT column.

Keywords: Gas Chromatography, Medical, Portable Instruments
Application Code: Biomedical
Methodology Code: Gas Chromatography
Background/Aim: MicroRNA 26a (Mir-26a) has been reported to modulate gene regulation influences the maintenance of metabolic homeostasis, particularly the states of obesity, thereby providing a potential link between miRNAs and nonalcoholic fatty liver disease (NAFLD). Methods: In the current study, experimental rats were fed a high-fat diet (HFD) for 6 and 16 weeks to establish a rat model of NAFLD, while control rats received standard chow. Serum and liver tissue was collected from all animals at 6 and 16 weeks of feeding. Biochemical parameters (cholesterol, TG, AST, ALT, NEFA) were determined. Hepatic lipid accumulation was estimated by Oil red O staining. Vectors encoding pre-Mir-26a (LV-26a) and an empty lentiviral vector (LV-Con) delivered approximately 2 x 10^7 transforming units of recombinant lentivirus were injected to mice through the tail vein. Serum IL-6 was measured by ELISA and miR-26a was detected by qRT-PCR. Results: Over the 16 weeks of feeding, body weight, liver lipids, serum triacylglycerol levels and ALT, AST aminotransferases activities were markedly elevated in the HFD group compared to the control group. In contrast, LV-26a-infected mice showed marked reduction of total liver weight, hepatic triglyceride deposition and serum ALT concentration when compared with LV-Con-treated mice. In addition, the in vitro experiments with HepG2 and Huh7 cells showed that mir-26a inhibits the expression of IL-6. Furthermore, the decreased total liver weight, hepatic triglyceride deposition and serum ALT concentration induced by Mir-26a was also completely abolished by IL-6 overexpression. Histopathological examination of hepatic tissue reinforced these results. Conclusion: Our findings suggest that mir-26a-IL-6 axis regulates the development of NAFLD in a rodent model. Therefore, serum miR-26a level is indeed useful for assessing early NAFLD and might be superior to clinical markers traditionally used to monitor hepatic disease.
Highly pathogenic avian influenza viruses, such as H5N1, have caused human infections since 1997 representing a significant public health hazard. The current rapid tests for influenza viruses have low sensitivities making them of limited use in the clinical setting. The importance of developing a rapid test that only utilizes easily obtained specimens, has rapid turn-around and is highly sensitive cannot be overstated.

Our goal has been the development of diagnostic tests for influenza A virus with increased sensitivity, easily obtained specimens, and rapid turn-around for point-of-care deployment. To reach those goals, we utilized an optical impedance spectroscopic technique based on a single-mode, electro-active, integrated optical waveguide (EA-IOW) platform, which is a technology that was previously pioneered by us to investigate electron-transfer processes of redox adsorbates with unprecedented sensitivity. In this effort we have targeted the hemagglutinin (HA) protein from the H5N1 influenza A virus to demonstrate the capabilities of the EA-IOW device for detection and quantification of bio-agents.

Our highly sensitive and selective sandwich immunoassay sensor for interrogation of the HA consisted of a monoclonal anti-H5 (H5N1) antibody (Ab) bound to the functionalized EA-IOW interface, followed by the immobilization of the HA and finally the adsorption of a polyclonal secondary Ab labeled with methylene blue (MB) dye. The experimental results successfully corroborated the transduction capability of the EA-IOW platform to detect the electron transfer events of MB-labeled Ab as different layers of the immunoassay were incorporated to the device surface. The detection limit of the HA was in the order of pico-molar range, which already surpasses several technologies currently being deployed and places us at the frontiers of the state-of-art.

**Keywords:** Biosensors, Immunoassay, Spectroelectrochemistry, Surface Analysis

**Application Code:** Biomedical

**Methodology Code:** Integrated Sensor Systems
Phthalates, classified as Endocrine Disrupters, are used as plasticizers to promote flexibility and durability of plastics. Therefore, these compounds can be found in numerous sources of exposure, especially children’s toys. Additionally, information related to this exposure and to the quality control of toys are still scarce in Brazil. In this sense, this study aimed to evaluate the presence of phthalates in toys sold in Brazil. For this proposal, a gas chromatography/mass spectrometric system was used for simultaneous determination of dimethyl phthalate (DMP), dibutyl phthalate (DBP), benzyl butyl phthalate (BBP), bis (2-ethylhexyl) phthalate (DEHP) in toys samples. These compounds were extracted using hexane and ultrasonic bath. Afterwards the quantification was performed by external calibration using internal standard. The method was validated to ensure that the results were within the acceptable limits. The detection and quantification limits were, respectively, 0.1 and 0.5 µg/mg for all phthalates. The calibration curves were linear with correlation coefficients higher than 0.99 in the range level of 0.5-4.0 µg/mg. Intra and inter-assay precision (relative standard deviation) were all ≤15% and accuracy did not exceed 15%. Recovery values for total phthalates were ranged within 89.8-103.7%. Furthermore, 40 toys samples were analysed. DEHP and DBP were the predominant phthalates found. The concentrations were higher than the allowed limit (0.1% w/w) for DEHP in 20 samples and DBP in 3 samples. Both DMP and BBP were found at low concentrations. The results showed that these toys have significant proportions of phthalates that can contribute to increase exposure risk in childhood.

Keywords: Gas Chromatography/Mass Spectrometry, Polymers & Plastics, Toxicology, Validation
Application Code: Clinical/Toxicology
Methodology Code: Gas Chromatography/Mass Spectrometry
An Assessment of Drugs Other Than Nicotine (DOTNs) in Electronic Cigarette Products

Electronic cigarettes (e-cigs) were developed as an alternate nicotine delivery system. As their popularity grew, so has the proliferation of internet sources promoting the use of drugs other than nicotine (DOTNs) in the e-cigs. DOTNs include natural and plant-based products, designer drugs, and traditional and non-traditional pharmaceuticals. We assessed 29 commercially available products used in or advertised as e-cig formulations that were suspected or labeled to contain DOTNs. These products were analyzed for drugs by Direct Analysis in Real Time AccuTOFTM Mass Spectrometry, Gas Chromatography Mass Spectrometry and Liquid Chromatography Tandem Mass Spectrometry. Solid phase micro-extraction technique was used in identifying the drugs in the aerosol produced by an e-cig.

Four samples were determined to contain the mitragynine and 7-hydroxymitragynine constituents of Kratom. Three samples were determined to contain the cannabimimetic MDMB-Fubinaca. Five products labeled as Blue Lotus flower, were determined to contained apomorphine and/or nuciferine. Seven samples labeled to contain herbal remedies such as poppy, catnip, and maca root and two labeled as vitamin were determined to contain no detectable DOTNs. Three samples were determined to contain caffeine and one, melatonin. Four samples, labeled to contain THC and/or CBD, were determined to contain the labeled cannabinoid contents as well as unlabeled cannabinoids. When present, the DOTNs were successfully aerosolized in an e-cig. These products demonstrate the proliferation of e-cigs as a drug delivery system for more than just nicotine.

This project was supported by NIJ (NIJ-2014-R2-CX-K010 & 2016-DN-BX-0150) and NIH (P30DA033934)
Abstract Text

Whole-exome sequencing identified several driver mutations that may underlie CLL heterogeneity. Mutations in the toll-like receptor (TLR)/MYD88 pathway, described in 4% of the CLL patients, are associated with IGHV mutated status and younger age at onset. TLRs are essential receptors of the innate immune system and are involved in B-cell activation. MYD88 is complexed with IRAK4 and IRAK1, leading to activation of NF-κB. We have analyzed TLR pathway in CLL cells and the possibility to target CLL cells with the IRAK4 inhibitor ND2158 (Nimbus Therapeutics). Gene expression profiling of 455 CLL cases revealed that TLR1, TLR10 and TLR7 were the most highly expressed TLRs in CLL cells, followed by TLR2 and TLR6. CLL samples with and without MYD88 mutations and mononuclear cells obtained from healthy donors were cultured in vitro in the presence of several doses of ND2158 (5 to 100 μM) for 24 and 48 hours. ND2158 induced a selective and dose-dependent cytotoxic effect in CLL cells compared to B and T cells from healthy donors. There were no significant differences in ND2158 cytotoxicity regarding the mutation status. By western blot we observed that ND2158 was able to downregulate the phosphorylation levels of IκB and STAT3. In vitro stimulation of TLRs pathway was performed with its agonists. Using the Luminex technique, an increase of cytokine secretion (MCP-1/CCL2, MIP-1α/CCL3, MIP-1β/CCL4) was observed when TLRs were stimulated. Basal levels of these cytokines were found to be higher in MYD88 mutated CLL cases than in CLL unmutated cases. Also an increase on the expression of B cell activation markers (CD86, CD40, CD25 and CD69) were observed after TLR stimulation. ND2158 effectively reduced cytokine secretion and the expression of these cell activation markers. Our findings support pharmacological inhibition of IRAK4 as a possible therapeutic strategy in CLL cells regardless MYD88 mutational status, indicating that B-cell intrinsic MYD88 signals have an important role in CLL cells.

Keywords: Biomedical
Application Code: Biomedical
Methodology Code: New Method
Cancer is a major cause of death worldwide according to the National Cancer Institute and early diagnosis is crucial to control disease status and evolution, moreover detection of cancer in early stages of the disease is of utmost importance for clinical diagnosis. Therefore, the development of biosensors has enormous potential to enhance the evolution of new diagnostic techniques with high detection sensitivity, specificity, and multiplexing capacity. In this work, we developed an integrated DNA aptamer-based biosensor to detect relevant biomarkers for prostate cancer: prostate-specific antigen (PSA), vascular endothelial growth factor and cell surface associated mucin 1 (MUC1). A thiolated DNA hairpin containing binding aptamer was conjugated with methylene blue (redox tag), and was immobilized on a gold electrode by self-assembly. Binding of specific protein to the aptamer induced the unfolding of aptamer hairpin, 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, Biomedical, Biosensors

**Application Code:** Biomedical

**Methodology Code:** Sensors
In this study, Raman/ROA, and infrared (IR) spectroscopic techniques were used for the characterization of different Hyaluronic acid (HA) products and their particular structure. HA is a linear polysaccharide formed from disaccharide units that contain N-acetylglucosamine and glucuronic acid. HA is currently used by plastic surgeons around the country as an antiaging and enhancement measure in noninvasive cosmetic procedures. The commercial forms of HA available come in a cross-linked form of the acid or its salt and some forms include small percentages of Lidocaine as anesthetic. Multiple Cross-linked HA samples were tested using Raman/ROA, and infrared (IR) spectroscopy to determine the structure and wavenumber of multiple HA components, as well as the Lidocaine presence in some of the samples. The samples were measured in hydrated form reason why water subtraction was required for further analysis. The infrared (IR) spectra was collected with a Bomem FTLA2000 FT-IR bench using a PIKE MIRacle single bounce diamond ATR accessory to confirm the secondary structure of the samples compared to our standards. Some of the collected results showed abnormalities in the spectra that were further analyzed using Raman/ROA. The results collected from Raman, ROA techniques using a BioTools ChiralRamanTM Raman Optical Activity (ROA) spectrometer which operates in the visible spectral region at 532 nm excitation to deep UV using a frequency doubled Ar+ laser with excitation at 229 nm to confirm the primary hypothesis formulated from the IR spectra about the spectra abnormalities and allow us to identify multiple errors in the cross-linking procedures of some commercial samples, as well as the consequences on this in the overall HA structure.

Keywords: Biomedical, Infrared and Raman, Spectroscopy
Application Code: Biomedical
Methodology Code: Biospectroscopy
Carbamate pesticides have been largely used worldwide because of their characteristics (broad biological activity spectrum, low bioaccumulation potential and short-term toxicity). Pirimicarb (PMC) is a carbamate pesticide mainly used to control aphids on vegetables, cereal and orchard crops. It is classified as “likely to be carcinogenic to humans” by the EPA [1], which demonstrate the necessity of an analytical method to monitor this carbamate pesticide in-field. Hence, a Differential Pulse Voltammetry (DPV) method was developed to monitor PMC using Boron-Doped Diamond (BDD) electrode pre-treated cathodically. The electrochemical study was initially performed evaluating the pH dependence with electrochemical signal (pH from 2.0 to 8.0). Depending of the pH value, the voltammograms exhibited four oxidation processes; the first two are pH dependent, while the last two are not. Each oxidation process undergoes single electron transfer. In light of the results, a mechanism for PMC electrochemical oxidation was proposed. The best DPV parameters (Step (E[sub]s[/sub]), Amplitude potential (E[sub]a[/sub])) and Scan rate (v) were evaluated using an experimental design approach (two levels and 3 parameters, 2([sup]3[/sup]/[sub]/[sup]/[sub])) and the optimized values were E[sub]s[/sub] = 13.13 mV, E[sub]a[/sub] = 131.8 mV and v = 20.89 mV s([sup]1[/sup]/[sub]/[sub]). Analytical curve under the best conditions ranged from 2.00 to 219 µmol L([sup]1[/sup]/[sub]) (R([sup]2[/sup]) = 0.9982) and limit of detection (LOD) was estimated as 1.24 µmol L([sup]1[/sup]/[sub]) (3 slope). The proposed method showed a relative standard deviation of 1.05% (n = 10) and the accuracy was evaluated by addition and recovery protocol in real river and tap water samples. The recoveries values ranged from 88.6 to 96.3%.

Acknowledgments: Brazilian funding agencies (Capes, CNPq and FAPESP).

Environmental Analysis of Pesticides, PPCPs, VOCs and other Organics

Efficient Sample Workflow from Extraction to Analysis for Pesticides Using US EPA 608/8081

The analysis of pesticides continues to be an important measurement to monitor contamination in many matrices that might contribute to human exposure, such as water, soil and food stuffs. Pesticides in water, including drinking, wastewater, and groundwater are the largest contributors and hence it is especially important to consider for a wide variety of current, banned and potential pesticides.

The goal is to perform these analyses with the most efficiency to minimize turnaround time and cost. The impact of two steps in the process will be evaluated in this work. The elimination of the solvent exchange step to replace dichloromethane (DCM) with hexane to be compatible with GC-ECD is time consuming, but necessary because the best extraction efficiency for a liquid-liquid extract is obtained with DCM. Using a solid phase extraction disk provides the same or better extraction and subsequently hexane can be used directly to elute the analyte from the disk. The drying of extracts using a membrane system rather than sodium sulfate eliminates a number of problems and helps to smooth the overall sample preparation and delivery of a clean sample to the chromatographic system for analysis.

The overall impact of these two changes to the traditional workflow will be explored and the value to different sized laboratories measured.

Keywords: Environmental/Water, GC, Solid Phase Extraction

Application Code: Environmental

Methodology Code: Gas Chromatography
Environmental Analysis of Pesticides, PPCPs, VOCs and other Organics

New Workflow for Identification of Multiclass Pesticides at <1 ppb Levels in Fish Samples by LC-MS/MS Using Enhanced Product Ion Spectrum

The identification and confirmation of organic contaminants by liquid chromatography-tandem mass spectrometry (LC-MS/MS) at not quantifiable levels in biological matrices is a major challenge with actual environmental concern. The confidence in such qualitative information for unambiguous identification is related to the selectivity and specificity provided by sensitive LC-MS platforms. A widely used approach for identification by LC-MS/MS is the retention time matching plus the ratio between the qualifier and quantifier multiple reaction monitoring (MRM) transitions. But at those challenging levels the fitting with deviation tolerances (±30%) led to the risk of false positive or false negative identifications if MRM ratio criteria is used.

In this work, we present the application of an alternative identification workflow to the classical MRM ratio to identify multiclass pesticide residues at sub [micro]g/kg levels in fish muscle tissue samples used for environmental biomonitoring. Original QuEChERS extracts of wild South American fish species are subjected to LC-MS/MS analysis using MRM and the so called MRM-IDA (Information Dependant Acquisition) mode for enhanced product ion (EPI) scanning available in QLIT (quadrupole – linear ion trap) analyzer. The reason of using these strategies is based on the fact that full scan MS/MS spectra contain a higher degree of structural information useful for confident identification. Discussion is given on identification and confirmation issues of agricultural pesticides at <1 ppb levels in real-world samples.

Keywords: Environmental, Environmental/Biological Samples, Ultratrace Analysis
Application Code: Environmental
Methodology Code: Liquid Chromatography/Mass Spectrometry
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 that are not fully accessed or understood by the current literature. In Southwest Illinois, there is a mixture of pristine, agricultural, suburban, and urban areas within a geographically small region presenting the opportunity to study a diverse source of PPCPs within a narrow variance of soil matrix. Both soil and water samples were collected from multiple sites of each type of area and analyzed for PPCP concentration. Sampling was completed during different seasons in order to minimize bias based on temperature and agricultural/recreational activities. Following collection, soil samples were processed using modified extraction protocols developed specifically for the PPCPs of interest. PPCPs were quantified from solution using liquid chromatography – multiple reaction monitoring – mass spectrometry following modified solid phase extraction protocols. The results indicated that there is correlation between the observed concentration in soil and in water. The levels of PPCPs also varied widely among the different environments that were analyzed lending speculation to the origin of some PPCPs.

Keywords: Environmental/Soils, Environmental/Water, Liquid Chromatography/Mass Spectroscopy, Pharmaceutical
Application Code: Environmental
Methodology Code: Liquid Chromatography/Mass Spectrometry
There are several methods available for the extraction and analysis of Steroid Hormones, Pharmaceutical and Personal Care Products (PPCP’s), and Pyrethroids in aqueous samples. However, very few procedures are available for extracting these compounds in more complex solid matrices such as sediments. Typical methods used are soxhlet extraction, Pressurized Liquid Extraction (PLE), ultrasonic, and microwave assisted extraction. These methods tend to take longer and consume significant amount of solvents. In 2003, a new extraction procedure called QuEChERS (Quick-Easy-Cheap-Effective-Rugged-and Safe) was introduced. It was originally developed to extract pesticides in food matrices but has since found applications in the environmental field.

Developed is a modified version of the QuEChERS method to extract natural and synthetic hormones, Pyrethroids, and Pharmaceutical and Personal Care Products from marine and river sediment samples followed by Liquid Chromatography-Tandem Mass Spectrometry (LC/MS/MS) analysis. The result is a rapid, simple, and efficient extraction and analysis with reporting limits in the low ng/g range. The use of the modified extraction and clean up method resulted in higher sample throughput, faster extraction times, and greatly reduced solvent consumption compared to conventional solid matrix extraction methods.

Keywords: Contamination, Environmental Analysis, HPLC, Mass Spectrometry
Application Code: Environmental
Methodology Code: Liquid Chromatography/Mass Spectrometry
Environmental Analysis of Pesticides, PPCPs, VOCs and other Organics

Solutions for the Determination and Stability of Bromomethane by Purge and Trap

Bromomethane stability has continually been a problem compound in environmental laboratories. This problem has been so pervasive that many purge and trap concentrator manufacturers have developed their own analytical trap designs in order to improve upon the linearity and stability of this compound. The Vocarb® 3000 trap has long been the ideal trap for the determination of volatile compounds, so while designing a new trap for the problem compounds can help with the analysis of some of the compounds sacrifices are made with others. Knowing this, an experimental study was performed in order to examine purge and trap parameters that would enhance the stability and linearity of Bromomethane using the Vocarb® 3000 trap.

Keywords: Environmental Analysis, GC-MS, Purge and Trap, Sampling
Application Code: Environmental
Methodology Code: Gas Chromatography/Mass Spectrometry
In the United States the Environmental Protection Agency requires purge and trap sampling for the extraction of volatile organic compounds. Purge and trap sampling is an exhaustive technique which aids in the detection of lower level contamination in environmental samples. In order to determine the compound pollution levels, a Gas Chromatograph (GC) coupled to a Mass Spectrometer (MS) for separation and analysis are commonly used. Since USEPA method 8260 was written, there have been many advancements in GC/MS analysis. Because of these advancements, detection of compounds of interest has gotten lower. In Europe and Canada, static headspace analysis is commonly used for volatile compound sampling. This study will examine the detection of volatile organic compounds in water using static headspace sampling.

Keywords: Environmental/Water, GC-MS, Headspace, Sampling
Application Code: Environmental
Methodology Code: Gas Chromatography/Mass Spectrometry
Classical Purge & Trap analysis for volatiles hasn’t really changed much over the last 40 years. Now a unique innovation in Autosamplers have improved the technique, by allowing the lab to switch between Purge & Trap Soil and Waters to Thermal Desorption. Literally running one by day and the other by night.

This poster presents data showing purge and trap soils and/or waters run for US EPA methods, followed by a quick switch over to Thermal Desorption to run EPA 325 for Benzene. This unique Autosampler allows environmental labs to streamline operations, running one set of methods during the day and the second at night.
Volatile organic compounds (VOCs) contamination in natural water is a major environmental issue due to their toxicity and various adverse effects on human health. Since the maximum levels of contaminants allowed for many VOCs are very low ([μg L\(^{-1}\)] or parts-per-billion in terms of mass), sensitive and quantitative analytical devices are in great needs for long-term monitoring and remediation applications.

We presents the design, assembly, and characterization of a fully automated portable gas chromatography coupled with a purge-and-trap system for sensitive and rapid field analysis of volatile organic compounds (VOCs) in water samples. The VOCs were first purged by helium gas into the micro-fabricated preconcentrator/injector and then injected into the downstream capillary column and photoionization detector for separation and detection. The purge-and-trap conditions were optimized to efficiently extract VOCs from water samples. The calibration of 6 VOCs with concentrations range from 1 [μg L\(^{-1}\)] to 500 [μg L\(^{-1}\)] showed excellent linearity (R\(^2\) > 0.99). Detection limits (3[σ]) of sub-[μg L\(^{-1}\)] (or sub-parts-per-billion level) were achieved, which are orders of magnitude lower than the maximum contaminant level (MCL) established by the US Environmental Protection Agency (EPA). Separation of 26 analytes (vapor pressure ranging from 0.087 Torr to 180 Torr) in a water sample in less than 15 minutes was also demonstrated. Finally, the optimized system was applied to field analysis of a groundwater sample in an environmental remediation site. The quantification results agreed well with those obtained by an analytical lab under standard analytical methods and instrument. Our system offers a lab-on-a-chip solution for sensitive and rapid water analysis with the EPA compliant sample collection method. It will have a wide range of applications in environmental monitoring, industries, and healthcare.

**Keywords:** Environmental/Water, Gas Chromatography, Purge and Trap, Thermal Desorption

**Application Code:** Environmental

**Methodology Code:** Gas Chromatography
Concern for the environmental and health effects from exposure to materials from leaking underground storage tanks has led to the development of analytical and sample preparation techniques by several states to address specific needs from their own geographical areas. One of the earliest of these was devised by the Massachusetts Department of Environmental Protection. This method is intended for either qualitative identification of total petroleum hydrocarbons (TPH) or for fractionation, detailed analysis, and quantification of both aliphatic and aromatic fractions from site samples of water or soil/sediment matrices [1].

This state method, as well as other similar state methods, presents difficult challenges to laboratories who need to accurately implement techniques employed by the method to effect desirable results and particularly difficult is the fractionation of the aliphatic and aromatic components. The 5 gram silica gel cartridge specified in the methods needs to be of high quality and consistency in order to achieve adequate separation of the two fractions with minimal interferences.

This work presents a look at the performance of a newly tuned EPH specific silica gel cartridge used to carry out this critical fractionation step required of these EPH specific methods. Several key attributes, such as minimal background extractables, maximum resolving power of aliphatic from aromatic components, and consistent moisture control will be evaluated for their effects on performance.

Reference:

Keywords: Environmental, Environmental Analysis, PAH, Sample Preparation
Application Code: Environmental
Methodology Code: Sampling and Sample Preparation
Decomposition kinetics of gaseous α-pinene with ozone under the coexisting nitrogen monoxide (NO) was studied. The decomposition experiments were conducted in a plastic bag under a dark condition at room temperature with the fixed initial concentration of 0.7 ppm for α-pinene and 0.4 ppm for ozone. The initial concentration of NO was changed in the range of 0 (no addition) to 0.6 ppm. The time changes of the concentrations of α-pinene was continuously monitored with the PTR-MS technique and that of ozone was also monitored with an ozone meter, and that of NO was monitored by an NOx meter. The decomposition rate of α-pinene was gradually decreased by increasing NO concentrations up to 0.6 ppm, and a sudden drop was observed at 0.9 ppm. Without nitrogen monoxide, the ozone concentration decreased gradually, corresponding to the dissociation of α-pinene. When initial concentration of nitrogen monoxide was low (0.1 to 0.6 ppm), the ozone concentration dropped rapidly and nitrogen monoxide was quickly converted to nitrogen dioxide at the initial 1 min, which can be attributed to a fast reaction of ozone with nitrogen monoxide. After that ozone concentration was lower than the case without nitrogen monoxide. When the initial concentration of nitrogen oxide was 0.9 ppm, the ozone concentration dropped suddenly at initial 1 min, and after that the ozone concentration was below the detection limit. These results suggest that not only ozone is involved in the decomposition of α-pinene but some other oxidative species such as radicals would react with α-pinene, depending on the initial concentration. The products of ozone oxidation was analyzed with GC-MS (gas phase) and with a particle analyzer, and found to depend on the nitrogen oxide concentration.
Monitoring marine top predators, primarily fish species, in open waters, provides important insights into marine ecosystems through the understanding of the population and community dynamics of fishes. Recently, biologging techniques involving electronic data-storage tags and acoustic transmitters have been increasingly used to understand migratory fish movements and behaviours. The number of tags, however, is normally limited due to costs, and the tag recovery rate is still low. In this study, therefore, to increase a recovery rate of data from marine top predators, we have been developing a new biologging system based on a variety of small, low-cost, large-data-capacity and multifunctional tags. The system consists of the following four development: (1) two types of archival tags (small-sized tags and customizable multifunctional tags), (2) the energy harvesting system installed in the tag, (3) the data receiving system onboard multi-platforms, and (4) the inter-individual communication system based on hydro-acoustic methods. Currently, we have started testing the developed system in open waters off Japan. In the presentation, we show the developed system and preliminary results obtained from the open water experiments. The new technology will overcome the bottleneck of conventional biologging techniques, and will lead to a breakthrough in marine ecosystem studies. This work has been supported by CREST, Japan Science and Technology Agency.
Anthropogenic increases in CO2 cause ocean acidification and global warming, declining calcium carbonate saturation, and reduced coral reef calcification. To evaluate responses of coral reefs and other marine ecosystem against the ocean acidification, monitoring of marine carbonate system is necessary, but total alkalinity (AT), the most important parameter for ocean acidification and calcification, has been measured by sampling large amount of seawater (> 100 ml) by titration. In this poster, we will report that we designed a continuous flow-through analyzer to measure seawater pH and AT with a small amount of sample (< 20 ml). Our system consists of four components: two lithium batteries, two ISFET and leak-free reference electrodes for pH and AT, a millimeter-scale of flow channel called “mini-TAS”, and two piezoelectric micro pumps. First, seawater sample is introduced to mini-TAS by piezoelectric micro pump, and pH of seawater sample is measured by ISFET and leak-free reference electrodes. Then HCl is added to the samples. AT can be measured by ISFET and leak-free reference electrodes with calibration of three different certified reference materials (A. Dickson, Scripps Institution of Oceanography). All data such as voltages for pH, AT, and temperature are logged by micro SD cards. Our system is now being applied for field use, and it will enable to monitor and evaluate the impact of ocean acidification on the marine carbonate chemistry and marine ecosystems at higher time and spatial resolutions.

Keywords: Environmental/Water, Lab-on-a-Chip/Microfluidics, Sensors
Application Code: Environmental
Methodology Code: Integrated Sensor Systems
Determinaton Of Molecular Mass 302 Polycyclic Aromatic Hydrocarbons in Standard Reference Material 1597A by Reverse-Phase Liquid Chromatography Coupled with Fluorescence Detection

Polycyclic aromatic hydrocarbons (PAHs) is a large class of organic environmental pollutants found worldwide. Due to their carcinogenicity and the vast assortment of natural and anthropogenic sources, it is of high importance to detect and identify these compounds through timely analytical methods. Current methodologies employ the use of gas chromatography-mass spectrometry and reversed-phase liquid chromatography (RPLC) coupled to an absorbance and/or fluorescence detector (FLD) for PAH identification. In this study, PAHs with molecular mass (MM) 302 Da are determined in standard reference material (SRM) 1597a using RPLC-FLD with 2D fluorescence spectra collection capabilities. The ability to collect 2D fluorescence spectra during a RPLC measurement allows for an additional method of PAH identification through the comparison of unique spectral features to that of respective PAH standards. Prior to RPLC-FLD analysis, a normal-phase liquid chromatography fractionation procedure using an aminopropyl (NH2) semi-prep LC column is used for sample cleanup to separate PAHs based on the number of aromatic carbons in the structure. The MM 302 PAHs were determined in three SRM 1597a fractions based on their retention times and unique spectral profiles.

H. V. Hayes and A. D. Campiglia acknowledges 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: Fluorescence, Liquid Chromatography, PAH
Application Code: Environmental
Methodology Code: Liquid Chromatography
Polycyclic aromatic compounds are a large class of environmental pollutants originating from a wide variety of natural and anthropogenic sources. In the past decades, polycyclic aromatic hydrocarbons (PAHs), which contain only carbon and hydrogen, are the most analyzed PAC in the world today. However, the incomplete combustion of organic matter leads to numerous heterocyclic compounds containing at least one heteroatom such as polycyclic aromatic sulfur heterocycles (PASHs). Because of the occurrence of PAHs and PASHs together in combustion-related samples, the analytical methods described in the literature requires multiple sample cleanup steps prior to determination via gas chromatography-mass spectrometry and liquid chromatography coupled to an absorbance and/or fluorescence detector. Normal-phase liquid chromatography (NPLC) using an aminopropyl (NH$_2$) semi-prep LC column is used to separate PAHs and PASHs into fractions based on the number of aromatic carbon in the structure. In this study, the retention behavior of 156 PAHs and 150 PASHs are investigated on an NH$_2$ analytical LC column. These retention data are the basis for designing the LC procedures for the fractionation of the complex mixtures of PAH and PASHs. Correlations are discussed in detail between the nonplanarity (thickness) of PAHs and PASHs and their retention behavior on the NH$_2$ stationary phase.

H. Hugh and A. D. Campiglia acknowledges 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, Liquid Chromatography, PAH, Prep Chromatography

**Application Code:** Environmental

**Methodology Code:** Liquid Chromatography
Over the last decade, UHPLC has been widely established as a powerful improvement over conventional HPLC technology. While being perceived as less rugged in the early days, UHPLC instrumentation has meanwhile achieved a high level of perfection, featuring a result precision where, however, minor deviations in the separation conditions can affect the analytical result. State of the art pump technology for instance provides a level of precision where even small pressure variations along the flow path translate into a retention time precision lower than the performance of the pump would enable. One crucial source for uncontrolled pressure deviations is the sample injection. The sample is normally aspirated and stored in the sample loop at ambient pressure, and then injected to the column by a switch of the injection valve. Thereby the sample is suddenly compressed, while the system pressure drops until the pump could recover the separation pressure. This has various detrimental impacts on the UHPLC separation, as it leads to a flow inconsistency, mechanical stress for the LC column bed, and to uncontrolled liquid expansion from the sample loop prior to sample aspiration.

This pressure shock can be mitigated by intelligent control mechanisms which enable to dampen the impact of the injection shock (SmartInject technology). SmartInject substantially improves retention time and peak area precision, next to an enhanced column lifetime by reducing the mechanical stress on the separation column during injection. This work exploits the capabilities of SmartInject technology and its impact on the named criteria in the performance range from conventional HPLC to cutting-edge UHPLC separations.

Keywords: High Throughput Chemical Analysis, HPLC, Liquid Chromatography, Sample Introduction
Application Code: High-Throughput Chemical Analysis
Methodology Code: Liquid Chromatography
Amino acids are the building blocks of life and essential for survival. Unfortunately, they are difficult to measure at low levels using High-Performance Liquid Chromatography (HPLC) due to their weak chromophores. Detector sensitivity can be improved by derivatizing the amino acids with OPA (o-phthalaldehyde) and FMOC (Fluorenylmethyloxycarbonyl chloride), but the challenge is that derivatizations are time-consuming and manually intensive. The solution is to “train” your autosampler to complete the derivatizations for you! This is done by programing the individual steps into the autosampler’s instruction set, drastically improving reproducibility and accuracy. We were successfully able to program both our older and newer generation HPLCs with the instructions to perform this derivatization, thus saving time and money while improving the consistency of the results. We demonstrate the programming, operation and results obtained while using this method for the determination of amino acids in a variety of samples including recovery sports drinks and fruit juices. In order to take full advantage of this automated sample prep “robot”, we developed a high-speed HPLC method for the analysis of the derivatized amino acids. We will also discuss the use of UV spectra generated from diode array detectors in order to help confirm the identity of various amino acids through the building and implementation of UV spectral libraries.
The importance of heparin, a sulfonated carbohydrate with a high negative charge, originates from its use as a blood thinner. Quality control of heparin to check for the contaminant oversulfated chondroitin sulfate (OSCS) is mandated by the FDA. Most ion exchange methods for heparin give broad peaks and take an excessive amount of time to conduct. Protamine, a small protein composed of about 70% arginine that gives it a highly positive charge, is considered an antidote to heparin. It is expected that a protamine coated cation exchange column could provide narrower peaks and shorter analysis times. Three test compounds (naphthalenesulfonic acid (NMS), naphthalenedisulfonic acid (NDS), and naphthalenetrisulfonic acid (NTS) were first chosen to understand the potential retention mechanism(s). At low ionic strength (0.01 M perchlorate), the anion exchange retention factor order of NMS < NDS < NTS is as expected. Interestingly, at higher ionic strength, there is a retention factor crossover at about 0.02 M perchlorate involving NMS and NDS. The peaks in each chromatogram were reproducible up to at least 0.05 M perchlorate. However, retention times for five replicate chromatograms of NMS, NDS, and NTS after using 0.1M sodium perchlorate showed a very slight downward trend. Protamine at the low ppm level has now been added to the mobile phase in order to ensure column stability and partially neutralize the heparin charge. Chromatograms of poly(styrenesulfonate) and heparin on the protamine coated column as a function of mobile phase ionic strength have shown quite narrow peaks at reasonable elution times.

Keywords: Biopharmaceutical, HPLC, Ion Exchange
Application Code: Bioanalytical
Methodology Code: Liquid Chromatography
FLOM corporation has been developed the pump and valve over 20 years. We developed the unique concept liquid feeding system controlling the system back pressure and flow rate simultaneously, is combined with the liquid feed pump, flow switching valve and controllable back pressure regulator. The back pressure regulator has a needle to open and close the flow path, a motor to move needle and a controller to feedback pressure. The range of pressure control in this system is 0 ~ 60 MPa. This system is able to achieve constant flow rate constant pressure even if there the pressure changeable element in the flow path. We show the system is applied to column packing as one of constant flow and constant pressure applications. The system is also available for the variable pressure application under constant flow, or the variable flow application under the constant pressure.

**Keywords:** Chromatography, HPLC, HPLC Columns, Liquid Chromatography

**Application Code:** Other

**Methodology Code:** Liquid Chromatography
Abstract Text

Chiral compounds have always proven to be very difficult compounds to enantiomerically separate. When it comes to most chemical and physical interactions, both forms of a chiral compound have the same characteristics, thus making their separation difficult. Chiral compounds have historically been separated via derivatization followed by chromatography. More recently, enantioselective chromatographic techniques have been developed. Typical chiral chromatography is done via HPLC in Normal or Reverse Phase conditions by a stationary phase functionalized with a chiral selector. These stationary phases range from chiral-functionalized silica, metal-ligand exchange, to polysaccharide phases. Recently, polysaccharide coated silica stationary phases have proven to be the most efficient in separating the widest variety of chiral compounds. They are versatile in their compatibility and are stable in acidic and basic conditions (pH 2-9) and can be run in normal, reverse, and polar organic solvents. While polysaccharide coated silica stationary phases are the most popular and the most widely successful columns in separating chiral compounds, there are still some compounds that prove difficult to separate, especially when limited to a specific pH range.

In this work we explore chiral selectivity beyond the typical pH limitations by employing high pH (9-12) for the chiral separation of amphetamine and its related molecules of interest on a new high pH stable polysaccharide based column. This work demonstrates a means to effectively separate the chiral forms of methamphetamine, amphetamine, derivatized amphetamines and other pharmaceutically relevant compounds.

Keywords: Chromatography, Clinical/Toxicology, HPLC
Application Code: Clinical/Toxicology
Methodology Code: Liquid Chromatography
High Performance Low Volume Static Mixer for Tough HPLC Applications

A revolutionary new inline static mixer has been developed to meet the exacting demands of liquid chromatography. The new static mixer accomplishes this goal via a novel 3D printing technology to create a unique 3D structure that achieves improved static mixing with the highest reduction in baseline ripple per unit of internal mixer volume. This mixer consists of interconnected 3D flow passageways having varying cross-sectional areas and path lengths as the fluid transverses across and through complex 3D geometric shapes. The mixing in the multitude of tortuous flow paths is coupled with turbulent flow and eddies to induce mixing.

Keywords: Chromatography, HPLC
Application Code: Process Analytical Chemistry
Methodology Code: Liquid Chromatography
As interest in cannabis grows, it is useful to consider various goals for the quantitative analysis of naturally occurring cannabinoids by HPLC. Potency determinations typically focus on THC-A and CBD, but there are myriad cannabinoids closely related to those targets. Proper attention to the analysis goals should dictate the parameters that guide method development. In this talk we present three different analysis goals and how they govern the chromatographic outcome.
Furocoumarins are a family of natural chemicals with phototoxic potential, commonly found in plant extracts and fragrances. The European Cosmetics Directive 76/768/EEC limits the total content of furocoumarins in cosmetics to 1 ppm. Current methods define “total content” based on a certain number of external standards (markers), overlooking other unknown furocoumarins. An accurate total content analysis is unpractical, but we aimed to develop a method to estimate total content of both known and unknown furocoumarins. As a family of chemicals, different furocoumarins have similar UV absorption spectrum (see Fig. 1). Therefore, we propose an HPLC method based on external markers and the use of DAD-UV to identify unknown furocoumarins. Eight markers were studied: Bergapten; Bergamottin; Imperatorin; Isoimperatorin; Isopimpinellin; Oxypeucedanin; Xanthotoxin and 8-Geranyloxypsoralen. Elution gradient comprised water, acetonitrile and tetrahydrofuran through a C18 column. We obtained well resolved peaks (purity factors all above 999.9%). Linearity proved to be satisfactory ($R^2$= 0.9977 to 0.9998) in the range of 0.1 µg/mL to 2.5 µg/mL). Intra-day precision was highly noticed (RSD= 0.0865% to 1.73%) and inter-day precision was further confirmed (RSD= 0.0539% to 3.92%), except for Bergamottin and 8-Geranyloxypsoralen. Quantification limits ranged from 345.2 pg to 3421.4 pg. Percent recovery was determined using a plant extract not known to contain furocoumarins, and results were acceptable (85.68% to 114.03%), except for Bergamottin and 8-Geranyloxypsoralen. Robustness was studied by changing flow rate, oven temperature and concentration of mobile phase. The method was fully validated for 6 markers. DAD-UV detector allows us to identify unknown furocoumarins in between the markers and estimate their content based on linear regression of equivalent markers.

Keywords: Consumer Products, Flavor/Essential Oil, HPLC, Validation
Application Code: Consumer Products
Methodology Code: Liquid Chromatography
Pesticides include all chemicals that are used to kill or control pests and often end up in drinking water. Accordingly, haloacetic acids (HAAs) are disinfection-byproducts formed in water when chlorine reacts with organic matter, and acrylamide is potentially used in water treatment, too. For decades the U.S. EPA has published official methods on how to prepare and analyze drinking water samples for contaminants. Even so, the overwhelming majority of these methods are antiquated and do not account for post-2007 rapid technological developments in analytical chemistry and instrumentation. To analyze acrylamide, HAAs, and pesticide residues in drinking water would require at least eight different EPA Methods comprising a variety of lengthy sample preparations and several separate expensive instruments. Initially, we endeavored to reduce expenses and time by performing these analyses using four different alternate test procedures. Still, lengthy sample preparation and instrument usage predominated the procedures. To substantially reduce costs and time we have developed and validated a performance-based method whereby we analyze acrylamide, five HAAs, and 31 pesticides in drinking water via direct large-volume injection onto LC-MS/MS after modest preparation in sample/methanol solution acidified with 5% formic acid. By combining these methods, we have reduced run time by 24%, overall preparation time by 90%, solvent costs by 25%, and overall supply costs by 64%. At The Coca-Cola Company, not only are our drinking water requirements more stringent than current regulations, but we also have passed 100% of all external and internal proficiency tests and audits for this method. The applicability to other labs is seamless, and over time the cost and time savings will compound resulting in true value creation.
Separation of ions or ionizable compounds using pure water as eluent and detecting them in a simple fashion has been an elusive goal. It has been known for some time that carbonic acid can be separated from strong acids by ion exclusion chromatography (ICE) using only water as the eluent. The practice of water ICE was shown feasible for very weak acids like silicate and borate with a dedicated element specific detector like an inductively coupled plasma mass spectrometer (ICPMS), but this is rarely practical in most laboratories. Direct conductometric detection is possible for carbonic acid but not especially sensitive due to its weak nature. Complex multistep ion replacement methods do not significantly improve its LOD. By using a permeative amine introduction device (Anal. Chem. 2016, 88, 2198-2204) as a conductometric developing agent, we demonstrate that a variety of weak acids (silicate, borate, arsenite, cyanide, carbonate, and sulfide) cannot only be separated on an ion exclusion column, they can be well detected (LODs 0.2-0.4 μM). We observe that the elution order is essentially the same as that on a nonfunctionalized poly(styrene-divinylbenzene) column and follows the reverse order of the polar surface area (PSA) of the analyte molecules. PSA values have been widely used to predict biological transport of pharmaceuticals across a membrane but never to predict chromatographic behavior. We demonstrate the application of the technique by measuring the silicate and borate depth profiles in the Pacific Ocean. The results of silicate show an excellent match with the results from a reference laboratory.
A permeative amine introduction device (PAID) is added after a conventional KOH eluent-suppressed conductometric anion chromatography (SCAC) system. The PAID can convert the suppressed eluates from the acid form to the corresponding ammonium salts (NR2H + HX → NR2H2+ + X). It allows very weak acids HX (pKa < 7.0) that cannot normally be detected by SCAC to be measured by a second conductivity detector following the PAID. Permeative reagent introduction can be performed without pumps, providing good mixing (baseline noise 0.8 nS/cm for 27 M diethylamine) with no dilution and low band dispersion (as small as 30 L). Diethylamine (DEA) was chosen as the amine source due to its high vapor pressure, low pKb value (3.0), low odor, and low toxicity. The eluite is thus detected against a low diethylammonium hydroxide (DEAOH) background (5 S/cm) as negative peak because the equivalent conductance of OH is greater than that of X. Reducing the background DEA concentration improves the detectability of traces of weak acids, but limits the maximum concentration of analyte acids that can be determined. A general concept of peak width based quantitation at a fixed height is proposed as a solution. The appearance of silicate in a glass container as a function of pH can be readily followed. The maximum silica level in high purity type 1 water is 50 nM (1.40 g/L Si), which is a measurement challenge. In the present work, 1 mL injection volume permits detection limits of 21 nM silicate, 3 nM taurine, 3 nM sulfide, and 13 nM cyanide.
Detection of small amounts of dissolved compounds with little or no absorption in the UV/Vis spectral range represents an analytical challenge even today. The differential refractometer is an established work-horse for the detection of alcohols, sugars, lipids or polymers in HPLC (high performance liquid chromatography) and GPC (gel permeation chromatography).

The KNAUER AZURA RID 2.1L is a highly competitive and sensitive refractive index detector. This device is ideal for fast and reliable routine analysis of non-UV absorbing substances. Improved safety features and enhanced diagnostics functions guarantee easy handling and minimal maintenance. A number of innovative features such as extended dynamic range make it unique on the today’s market.

The AZURA RID 2.1L is available as a kit specifically designed for OEM integration and business. The kit has a modular set-up, multiple interfaces and is compatible with most Chromatography Data Systems. This device can be easily adapted and fitted into customized housings to fulfill specific market requirements. A number of different control options are supported: software drivers, cable less user interface or integration into customers’ software.

Keywords: Detector, HPLC, HPLC Detection, Liquid Chromatography
Application Code: General Interest
Methodology Code: Liquid Chromatography
Valves are important accessories and should easily adapt to applications and chromatographic systems. They are widely used in a number of different applications like injection, column selection or further switching task. Manual control by lever is possible as well as automatic operation using a valve drive.

The KNAUER portfolio covers valves for analytical and preparative chromatographic applications. Due to flexibility in material, valves can be used for standard HPLC tasks, purification tasks, FPLC and dosing applications with different eluents, and even at high temperature. KNAUER’s valve drive “Valve Unifier” fits to all KNAUER valves and thereby supports a wide range of HPLC applications. Valves are recognized by novel RFID technology guaranteeing easy handling and maintenance.

Due to its smart design, the Valve Unifier fits to any kind of system solution or application. The device can be integrated into housings with compact dimensions. OEM integration is entirely supported based on the possibility of customization and the availability as kit. KNAUER provides a variety of control options: drivers for many chromatography software packages, analogue control or user friendly display control.

Keywords: Chromatography, High Temperature, Liquid Chromatography, Sample Introduction
Application Code: General Interest
Methodology Code: Liquid Chromatography
Determination of Chelator and Cu2+ Concentration in Liposomal Formulations Using High Performance Liquid Chromatography with UV Absorbance Detection and Atomic Absorption Spectroscopy

Purpose: To develop an HPLC/UV method for the determination of chelator and an atomic absorption (AA) spectroscopy method for the determination of Cu2+. The selectivity of the HPLC method allowed for the separation of the chelator from a liposome matrix and citrate buffer. The AA method could then be used to confirm the concentration of Cu2+ in the aqueous formulations.

Method: An Agilent 1100 HPLC coupled with a variable wavelength detector was used for the quantitation of a chelator compound in aqueous formulations containing excipient liposomes and Cu2+ in citrate buffer. Reproducible chromatography was achieved using a Synergi Polar-RP, 250 x 4.6 mm, 4-µm particle size column maintained at a temperature of 25°C. A gradient method was devised using two mobile phases: 0.1% trifluoroacetic acid in de-ionized water, and acetonitrile. A Perkin Elmer Analyst 800 AA Spectrometer equipped with a THGA furnace was used for the quantitation of Cu2+ in formulations containing chelator and liposomes in citrate buffer. Reproducible absorbances were obtained using a Perkin Elmer Lumina Hollow Cathode Lamp for Copper and a temperature gradient.

Results: The HPLC method was validated from 0.100 to 5.00 µg/mL. In addition, a quality control sample at 9.30 µg/mL showed inter-session variability of 1.5 to 7.7% RSD and inter-session accuracy ranging from 2.5-8.1% RE. The AA method demonstrated linearity from 10.0 to 50.0 ng/mL. In addition, a quality control sample at 377 ng/mL showed inter-session variability of 1.3 to 18% RSD and inter-session accuracy ranging from 7.7 to 22% RE.

Conclusion: An accurate method was developed and validated for the quantitation of the chelator and chelator-copper complex using HPLC/UV. A complimentary method was developed to determine the total concentration of Cu2+ in the liposomal formulations.

Keywords: Agricultural, Elemental Analysis, HPLC, HPLC Columns
Application Code: Environmental
Methodology Code: Liquid Chromatography
The analysis of isomeric impurities and structurally similar compounds are often a great analytical challenge due to the similarity in structures and chemical properties. Although most compounds have similar structural properties, they exhibit differences in biological activities such as pharmacology, toxicology, pharmacokinetics, and metabolism etc.

Undesired isomeric impurities in pharmaceutical products do not offer any therapeutic benefits for the patient and are sometimes toxic. Impurity control in pharmaceutical process scale up requires an understanding on the formation, fate, and purge throughout the manufacturing process. Orthogonal analytical approaches to quantitate and identify structurally similar compounds are highly critical.

Heart-cutting two-dimensional liquid chromatography (2D-LC) utilizing a reversed-phase achiral HPLC method in the first dimension with time-triggered fraction collection and subsequent chiral chromatography separation in the second dimension. This research demonstrates complete resolution of complex mixtures of stereoisomers and structurally similar compounds is one such orthogonal approach.

A 2D-UHPLC method was developed to separate 10 stereoisomers and structurally similar mixtures. The final chromatographic separation was achieved on Poroshell C18 achiral column in the first dimension. Our in-house customized 2D-LC system gives us the flexibility to analyze the collected fractions with multiple columns including Chiralpak IE-3 and Chiralcel OJ-3 chiral stationary phase in the second dimension. The heart-cutting 2D-LC approach has been used to achieve resolution of complex mixtures that otherwise could not be achieved using a single column. For synthetic process development, 2D-LC is an important tool to inform and direct process control.

Keywords: Chiral Separations, HPLC, Liquid Chromatography, Pharmaceutical
Application Code: Process Analytical Chemistry
Methodology Code: Liquid Chromatography
Ultra Strong Trapping of VEGF by Graphene Oxide: Anti-Angiogenesis Application

Angiogenesis is the process of formation of new blood vessels, which is essential to human biology, and also plays a crucial role in several pathologies such as tumor growth and metastasis, exudative age-related macular degeneration, and ischemia. Vascular endothelial growth factor (VEGF), in particular, VEGF-A165 is the most important pro-angiogenic factor for angiogenesis. Thus, blocking the interaction between VEGFs and their receptors is considered an effective anti-angiogenic strategy. We demonstrate for that first time that bovine serum albumin-capped graphene oxide (BSA-\text{GO}) exhibits high stability in physiological saline solution and possesses ultrastrong binding affinity towards VEGF-A165 \((K_d \approx 3 \times 10^{-12} \text{M})\), which is at least five orders of magnitude stronger than that of high-abundant plasma proteins such as human serum albumin, fibrinogen, transferrin, and immunoglobulin G. Due to the surprising binding specificity of BSA-\text{GO} for VEGF-A165 in complex plasma fluid, we have also studied the anti-angiogenic effects in vitro and in vivo. Results show that BSA-\text{GO} not only effectively inhibits the proliferation, migration and tube formation of human umbilical vein endothelial cells, but also strongly disturbs the physiological process of angiogenesis in chick chorioallantoic membrane and blocks VEGF-A165-induced blood vessel formation in rabbit corneal neovascularization. Our findings indicate that GO nanomaterials can potentially act as therapeutic anti-angiogenic agents via ultrastrong VEGF adsorption and its activity suppression.

Keywords: Biomedical, Biopharmaceutical, Drugs, Medical
Application Code: Biomedical
Methodology Code: Biospectroscopy
**Session Title:** Others  
**Abstract Title:** Is Tattoo Ink Safe? Analysis of Polycyclic Aromatic Hydrocarbons (PAHs) in Tattoo Ink by GC/MS  
**Primary Author:** Ramkumar Dhandapani  
**Phenomenex**  
**Co-Author(s):** Kristen Parnell, Matthew Trass, Sean Orlowicz, Timothy Anderson

**Abstract Text:**  
Tattoos have been popular in cultures around the world for centuries. In recent years, however, modern ink has come under scrutiny due to health concerns related to the purity of its contents. Polycyclic aromatic hydrocarbons (PAHs) in particular are listed as human carcinogens by the International Agency for Research on Cancer (IARC), and can form during ink production. Additionally, tattoo ink contains azo dyes that can form byproducts or break down into hazardous substances that cause allergic reactions or hypersensitivity; black and red dyes in particular are usually polyaromatic azo dyes, which can break down into individual polyaromatic amines. In the present study, we explore methods for testing of impurities in tattoo ink. Techniques for preparation and extraction of analytes from tattoo dye followed by analysis by GC/MS are presented. PAH separations is conducted using a Zebron ZB-5MSplus GC column, which provided an inert phase for improved peak shapes. Following analysis, techniques for further optimizing GC method parameters are discussed.

**Keywords:** Gas Chromatography, Gas Chromatography/Mass Spectrometry, PAH  
**Application Code:** Safety  
**Methodology Code:** Gas Chromatography/Mass Spectrometry
Ion mobility spectrometry (IMS) has proven itself as a powerful and efficient method for real-time and highly sensitive detection of drugs, explosives and chemical warfare agents, etc. However, the relatively low resolution (about 30) places the commercialized IMS equipment at the risk of high false alarm rate, due to the peak overlaps in the IMS spectra, especially when the mobility (K0) difference of two analyte ions is small. In traditional drift time IMS, the IMS curve can be approximately fitted with Gaussian peak when the gate pulse width not more than 0.2ms and Vdtg< 2 [diaresis (DLtd)] are satisfied. The relationship between the Gaussian peak parameters and the zero crossing point and maximum value of the first and second order Gaussian derivatives is investigated. The overlapping peaks in IMS spectra can then be resolved according to these relations. As a result, the achieved peak position error is less than 2%, and the full width at half maximum (FWHM) and intensity of IMS peaks can be obtained simultaneously. This method has been tested by theoretical simulation and experiments on a home-made IMS, respectively. It is demonstrated that this Gaussian differential method to be a promising way for improving the recognition accuracy rate of IMS.
Uncertainty evaluation is a very important stage for chemical measurements, involving plenty of statistical data processing. During the stage, the function of Excel is poor, while the using of Matlab, SAS and SPSS is too hard. By the guidance document of ISO/IEC 98-3:2008 Guide to the Expression of Uncertainty and related technical norms, we constructed the common models for chemical uncertainty evaluation. On the basis of theory study, we have developed software system with good stability and expansibility for uncertainty evaluation in chemical measurement by new software techniques. By using the system, analyzer can focus on setting up the model of the uncertainty evaluation and the data processing will be more easier and quicker.

The function of the system includes core service, service management and users' interfaces. Core service consists of basic syntax, basic functions, model templates and testing data library. Basic syntax and functions are type of variables, judge clauses, math functions and statistical functions, which can be used as needed by introduction. Users can design uncertainty evaluation models and then publish them. The published models will be referenced by others. During the design of models, users can define process procedure, input variables, function models, sensitivity coefficient calculation, uncertainty calculation freely. Users can select model template according to their own testing procedure. Service management is used to maintain the system. Other systems can invoke the functions by users' interfaces.
In an untargeted metabolomic study the search for biomarker molecules serves to answer many questions, for which there is a need to learn the identity of these metabolites. Several dozen metabolites are normally detected by NMR analysis of biofluids in measurable quantities, where the spectra can show about a few hundred peaks.

The identification of compounds is normally done with the aid of commercial software packages containing their own databases, by literature search, and/or by searches in public databases by lists of chemical shifts. The input for the database query can be improved using STOCSY, which is attainable given the amount of data collected for the multivariate data analysis.

Despite its usefulness, the STOCSY analysis is tedious and cumbersome, normally obtaining a trace by selecting a driver peak to find peaks highly correlated to it, and it is performed in a trace-by-trace fashion over all peaks of interest. Here we present a methodology developed to reduce the analysis time by increasing information recovery applying further statistical analysis on the “information redundant” STOCSY correlation matrix (all peaks included), yielding lists of peaks for database queries that produce more reliable hits during identification.

The methodology adds an automated step after the correlation matrix calculation to group traces from different driver peaks based on their similarity. As the STOCSY tool suffers from overlapped peaks, good alternatives to 1D 1H spectra are its use on 1D 13C spectra, 1D projections from 1H 2D J-Resolved spectra, and small-size data matrices like in a “spectrum-to-spreadsheet” procedure.

Keywords: Database, Identification, Metabolomics, Metabonomics
Application Code: Genomics, Proteomics and Other ‘Omics
Methodology Code: Data Analysis and Manipulation
**Abstract Text**

Pulp properties such as Kappa number are closely linked to the quality of final products. Determination of Kappa number and other parameters can improve process control to maximize yield and minimize costs associated with energy, chemicals, and waste disposal. Conventional methods of pulp analysis are destructive, require several time-consuming steps, and produce chemical waste. We demonstrate that near-infrared (NIR) spectroscopy can be used for rapid determination (30 seconds) of Kappa number and other pulp parameters while eliminating waste. Using chemometric techniques, we are able to correlate a variety of changes in NIR spectra to pulp properties. The correlations are validated via the standard errors of calibration, cross-validation, and prediction.

**Keywords:** Calibration, Chemometrics, Near Infrared, Paper/Pulp

**Application Code:** Environmental

**Methodology Code:** Near Infrared
Introducing Undergraduate Chemists to Chemometrics, PART 2: Performing Outlier Rejection Tests, and Streamlining the NIPALS Algorithm

This presentation is a continuation of work initiated by the author to introduce undergraduates majoring in chemistry, and also biology and other technical fields, to the field of chemometrics. With the widespread availability of instruments capable of collecting enormous amounts of data, and the software capable of analyzing that data toward analyte quantitation, it is highly important for undergraduate chemists to know and understand the principles and operations of various chemometric methods. Additionally, undergraduate chemists need to learn and understand various statistical tools (which are part of the field of chemometrics, too) for such data analysis tasks as rejection of suspect outlier data and results, among others. Software packages, e.g., Microsoft® Excel™ and MATLAB®, facilitate the performance of the plethora of mathematical and statistical methods for data and results analysis – and undergraduate chemists need to learn these tools as well.

In this paper, the author will present some handy techniques for setting up and implementing Microsoft® Excel™ worksheets for performance of outlier rejection tests, e.g., the Dixon Q, Grubbs G, and modified Thompson tau tests. Background on the outlier rejection tests will be presented, and the advantages and disadvantages of each test will be discussed. Specific examples utilizing synthetic and real data will be presented to illustrate how Excel™ may be used for each outlier rejection test. Also, the author will present and discuss attempts to streamline the NIPALS (nonlinear iterative partial least squares) algorithms for principal component analysis (PCA) and partial least squares regression (PLSR), in order to alleviate some of the tedium associated with performing such operations in the Excel™ environment. The significance of using Excel™ to illustrate the operation of NIPALS for PCA and PLSR, along with some background on PCA and PLSR, will be presented and discussed.

Keywords: Calibration, Chemometrics, Statistical Data Analysis, Teaching/Education
Application Code: Other
Methodology Code: Chemometrics
With the growing global population, the need to increase crop output and the nutritional value in the plants is becoming more important. Fertilizer is used to enhance the nutrient content of soils both to expedite plant growth and add nutritional content to the plants. While nutrients can be both organic and inorganic in nature, different techniques are required to analyze these types of compounds. When monitoring inorganic components, a variety of techniques can be used: flame AA, ICP-OES, and ICP-MS, each providing their own advantages. Given that the element nutrient content of fertilizers is fairly high and the number of elements limited, ICP-OES offers a good compromise between speed of analysis, sensitivity, cost, and ease of use. This work will focus on the analysis of nutrient elements in fertilizers using ICP-OES.
Different parts of Moringa oleifera plant have been advocated as an outstanding source of highly digestible protein, Ca, Fe, Vitamin A, C and E, β-carotene, amino acids and polyphenolics. Malnutrition is endemic to most developing and underdeveloped countries and Moringa holds potential to alleviating the problem of under nourishment. South African provinces engaged in production of Moringa, viz. Limpopo, KwaZulu-Natal and Mpumalanga are those that are also highly affected by poverty and malnutrition. Climatic conditions and farming practices vary significantly and may have an effect on the profile of nutrients, secondary metabolites of the leaves and possibly the antioxidant capacity of the leaf extracts of M. oleifera. Leaves were collected from trees in farms located in Limpopo (LP) and Mpumalanga (MP) provinces. Dried leaves were mineralized followed by analysis using ICP-OES for determination of nutrients. Secondary metabolites profile of the leaves were determined using FTIR and the antioxidants activities were determined using the DPPH assay. The effect of heat on phytochemicals was evaluated by placing leaves in a drying oven at 60°C for a period of 3, 6 and 9 months. Significantly high levels of macronutrients (mg/kg) Mg (>5000), K (>10000), Ca (>14000), P (>2000), S (>9000) and I (>3000) were observed in M. oleifera leaves collected from LP and MP. The antioxidant activity of Moringa leaf extracts compared to ascorbic acid (AA) followed the trend AA>LP>MP. The FTIR spectra of the leaves from LP and MP were very similar and showed two broad bands at 3280 cm⁻¹ (OH) and 2918 cm⁻¹ (C-H), medium and weak bands at 1613 cm⁻¹ and 1412 cm⁻¹ (C=C). Increased heat, over time (3 months < 6 months < 9 months) seemed to increase the antioxidant activity and the macro and micro nutrients in M. oleifera leaves. Significant gains in phenolics and antioxidant activity of ginger were also reported by Chumroenphat et al. (2011) during oven drying at 70°C.

Keywords: Analysis, ICP, Vibrational Spectroscopy
Application Code: Agriculture
Methodology Code: Atomic Spectroscopy/Elemental Analysis
Gas adsorption is used to analyze morphology of powder and porous materials including surface area, pore size, pore volume and porosity. These structure properties are closely related to materials performance in industrial processes and products. It has been a challenge to analyze different microporous materials with various porous structure accurately and efficiently. Previous work focused on analysis with N2 adsorption at liquid nitrogen temperature. Analyzing microporous materials with alternative gases and at alternative temperatures can extend application to various types of samples with different kinetics. In this study, we systematically characterize different powders and porous materials with focus on variously structured activated carbon materials using different gases at different temperatures to understand the morphology features and application conditions of different alternative gases. In addition, we understand and evaluate the significance of morphology difference across different materials based on statistical analysis.

Keywords: Characterization, Gas, Material Science, Statistical Data Analysis
Application Code: General Interest
Methodology Code: Physical Measurements
By using light as trigger it is possible to study many biological processes, such as the activity of genes, proteins and other molecules, with precise spatiotemporal control. Caged compounds, where biologically active molecules are generated from an inert precursor upon laser photolysis, offer the potential to initiate such biological reactions with high temporal resolution. As light acts as the trigger for cleaving the protecting group the ‘caging’ technique provides a number of advantages as it can be intracellular, rapid and controlled in a quantitative manner. We are developing caging strategies to study the catalytic cycle of a number of enzyme systems, such as nitric oxide synthase and ethanolamine ammonia lyase. These include the use of caged substrates, caged electrons and the possibility of caging the enzyme itself. In addition, we are developing a novel freeze-quench instrument to study these reactions, which combines rapid mixing and flashing capabilities. Reaction intermediates will be trapped at low temperatures and will be analysed by using electron paramagnetic resonance (EPR) spectroscopy to identify the involvement of any radical species during catalysis. The combination of rapid freeze-quench (RFQ) followed by EPR analysis provides the ideal approach to kinetically trap and spectroscopically characterise these transient radical species. In a typical RFQ experiment, two reagent solutions are delivered to the mixer via two syringes driven by a pneumatic actuator or stepper motor. The new mixed solution is then sprayed into a cryogenic liquid or surface and the frozen sample is then collected and packed into an EPR tube for analysis. In our work we are developing a novel RFQ instrument which combines the freeze-quench technology with flashing capabilities to enable the studies of both thermally-activated and light-activated biological reactions. This instrument also uses a new rotating plate design based on magnetic coupling.

Keywords: Analysis, Electron Spectroscopy, Ultra Fast Spectroscopy, UV-VIS Absorbance/Luminescence
Application Code: Other
Methodology Code: New Method
A recent study done shows that within 24 hours, carboxyl functionalized multiwalled carbon nanotubes (MWCNT) induce centrosome, mitotic spindle and chromosome number abnormalities in airway epithelial cells. Covalent modification of MWCNT with carboxylic acid, via acid washing, alters the carbon nanotubes solubility, length and surface. It is postulated that changes in physiological characteristics greatly affect the toxicity of MWCNT nanotubes in a living system. Therefore, with the increased use of functionalized carbon nanotubes in manufacturing as reinforced polymers and plastic composites it is important to rapidly determine physiological changes as it relates to molecular mechanisms of toxicity. To evaluate functionalized MWCNT an innovative analytical technique is utilized to quantify the effect of acid washing conditions on the molecular binding affinity to a model peptide. This label-free capillary electrophoresis technique is used to quantify changes in MWCNT carboxylation with a binding probe. The capillary electrophoresis technique was ability to differentiate carbon nanotubes acid washed for various amounts of time. This powerful tool also has the ability to differentiate carbon nanotube manufactured by different companies and acid washing techniques. Zeta potential measurements show a no change in surface charge as a function of acid washing, thus indicating that capillary electrophoresis is a viable method for analyzing physical changes in addition to elucidating toxicity at a molecular level.
**Abstract Title**: Improved Method for Pu(VI) by Chemometric Analysis of High-Quality Absorbance Measurements

**Abstract Text**

The shielded $\text{f-f}$ transition of Pu(VI) at ~830 nm is a favorite for absorption measurements due to its high molar absorptivity, which is at least 15x greater than any other transition for stable Pu oxidation states. Standard measurement methods for Pu-bearing nitric acid solutions, such as ISO 9463, feature quantitative oxidation to the VI state, usually with Ce(IV). However, the absorptivity and position of the peak are dependent on acidity and temperature. The published methods, which rely solely on measurement of the peak absorbance, require tight control of these quantities to obtain acceptable uncertainties for materials accountability measurements. Such control is not feasible for solutions from Pu processing streams, where acidities may range from <1 to >8 M and are often not determined with the desired accuracy to guide the redox chemistry. We have developed an updated analysis method based on a self-calibrating, high resolution diode array spectrophotometer and partial least-squares data analysis that eliminates these dependencies, improves method robustness and dynamic range, and maintains high measurement precision. These improvements should promote increased use of the method for process control and accountability measurements in nuclear materials processing facilities.

**Keywords**: Chemometrics, Nuclear Analytical Applications, Process Monitoring, UV-VIS Absorbance/Luminescence

**Application Code**: Nuclear

**Methodology Code**: UV/VIS
Electroplated Platinum Electrodes for Educational Purposes

Platinum electrodes are needed in several educational experiments since they are typically used as indicator electrode in redox titrations, construction of Clarke Oxygen sensors, amperometric sensors and cyclic voltammetry experiments. Moreover, platinum electrodes are commonly used as counter electrodes. Bulk platinum electrodes are commercially available at high cost and are frequently not available to every teaching lab and to some researchers as well. Platinized titanium electrodes are also commercially available but they are mainly fabricated for industrial purposes. In this work, we present the fabrication of stainless steel substrate electrodes, surface preparation and the two-step electroplating of bright and adherent platinum layer stainless steel substrates of various shapes. Disc electrodes in PEEK sleeves are prepared and critically compared to the commercial analogs based on bulk platinum electrodes. The versatility of the electroplated platinum/SS electrodes and their successful application in potentiometric, amperometric, and cyclic voltammetric experiments as well as detectors in flow injection analysis will be presented.

Keywords: Education, Electrochemistry, Electrodes
Application Code: Other
Methodology Code: Electrochemistry
Proteins are the main functional components in various food products and have ability to support dietary needs. Protein content is an important quality parameter in terms of price, nutritional value and labeling of various cereal samples. It has a crucial role in determining the textural, sensory and nutritional properties of the products. Therefore, determination of protein is an important quantitative analysis in terms of quality control, accurate nutrition labeling, pricing, functional property investigation and biological activity determination, which makes it a subject of both economic and social interest. However, conventional analysis methods, namely Kjeldahl and Dumas have major drawbacks such as long analysis time, titration mistakes, carrier gas dependence with high purity, and relatively low accuracy and precision. For this reason, there is an urgent need for rapid, reliable and environmentally friendly technologies for protein analysis. The present study aims to develop a new method for protein analysis in wheat flour and whole meal by using laser induced breakdown spectroscopy (LIBS), which is a multi-elemental, fast and simple spectroscopic method. Unlike the Kjeldahl and Dumas method, it has the potential to analyze high number of samples in a considerably short time. In the study, nitrogen peaks in the LIBS spectra of wheat flour and whole meal samples with different protein contents were correlated with results of standard Dumas method with the aid of chemometric methods. Calibration graph showed good linearity with the protein content between 7.9-20.9%, and 0.992 coefficient of determination (R2). Limit of detection (LOD) and relative standard deviation (RSD) for protein analysis in wheat flour and whole wheat meal was calculated 0.26% and 4%, respectively. The results indicated that LIBS is a promising and reliable method with high sensitivity for routine protein analysis in wheat flour and whole meal samples.
The methanizer has been around for decades. The methanizer consists of a heated catalyst which transforms CO as well as CO2 into methane to allow simultaneous detection along with hydrocarbons using an FID in conjunction with the appropriate chromatography. CO and CO2 are ubiquitous both in the atmosphere as well as in many industrial processes, which makes this an important analytical technique.

The methanizer can be used to improve the chromatography of these compounds by judicious use of parameters and component selection for FID, TCD and perhaps other detectors as well. A "real world" application will be used to demonstrate.

Keywords: Environmental/Air, Gas Chromatography, GC Detectors, Specialty Gas Analysis

Application Code: General Interest
Methodology Code: Gas Chromatography
How Scientific Companies use the Talent Supply Chain Management Model to Link Human Capital to Business Needs to Increase Productivity and Efficiency

Just like the conventional supply chain model, talent supply chain management (TSCM) is an adaptable process of mobilizing and engaging resources on an as-needed basis, creating a lean, streamlined system of input and output. Because it offers oversight as to where talent is sourced, how it’s engaged, and how it performs, TSCM allows companies to align their talent strategies with organizational objectives, optimize their workforces, and maximize the potential of their human capital—all while minimizing superfluous and inefficient processes. By engaging vendors and labor in a manner that’s in line with organizational goals, TSCM in effect creates an integrated or holistic system in which each part of the supply chain supports the rest. TSCM offers companies significant competitive advantages, including:

• Enhanced workforce planning.
• Oversight.
• Predictive capability.
• Risk mitigation.
• Access to a broader talent pool

Learning Objective 1:
Educate attendees on how to use the TSCM model to minimize risk associated in human capital when outsourcing project work: Risk mitigation. TSCM provides complete oversight as to sourcing and deployment of talent. At the same time, it yields data about quality. This enables companies to establish robust IP protection and risk mitigation policies and processes that are integrated with those of their vendors.

Learning Objective 2:
Workforce Planning Insight. The issue is often overlooked. We will present the most effective and creative way to manage attendees’ organization’s talent asset by evaluating all possible sources of talent in a holistic manner. By knowing how to engage workers, companies are better equipped to make sound decisions that are aligned with their overall objectives. We will also cover talent strategy that provides data about the deployment and performance of talent. When carefully analyzed and measured against industry best practices can provide a roadmap for your organizational growth.
A Reverse Intensity Correction method is developed for spectral library search to correct for instrument response without the side effect of magnifying the noise in the low responsivity region of test spectra. Instead of applying relative intensity correction to the sample test spectra to match the standardized library spectra, a reverse intensity correction is applied to the standardized library spectra to match the uncorrected sample spectrum. This simple procedural change improves library search performance, especially for dispersive CCD Raman analyzers using NIR excitations, where the instrument response often varies greatly across the spectral range, and SNR in the low responsivity regions is typically poor.
Integration of Multiple Instruments in a Chemistry Studio Classroom

Truman College, one of the City Colleges of Chicago, maintains two 32-seat chemistry “studio classrooms”. These are combined classroom/laboratory spaces adjoining an instrument room with IR, NMR, GC-MS, AA, microwave plasma atomic emission (MP-AES), digital video melting point apparatus, and a 3D printer. The studios exhibit multiple synergies that promote student learning in the laboratory. At the core, a hardware-based video distribution system allows the instructor to transmit any computer display simultaneously on each of the 16 student computers. We remotely connect to any instrument listed above, and transmit the screen to an interactive whiteboard and to every student, so that everyone has a “front seat”. Students can write on the live data projected on the whiteboard, enhancing our discussion of instrument operation and data analysis. Additionally, live video of melting point determination, sample preparation via document camera, and microscope images (e.g. of SPME fibers), further engage students with rich visual data. Maintaining this system requires constant vigilance because each instrument has its own software upgrade schedule. Operating system upgrades, monitor resolution issues, and institution-imposed network restrictions are also challenges we must overcome. Our poster describes the design, operation, and maintenance of this integrated teaching laboratory.

Keywords: Education, FTIR, GC-MS, NMR
Application Code: Laboratory Management
Methodology Code: Education/Teaching

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Date: Tuesday, March 07, 2017 - Morning
Time: Room: Exposition Floor, Aisle 2500-2600
Microcavity enhanced Raman sensing offers the tantalizing possibility of using non-destructive sensing methods to measure trace level components in both gas and liquid phase systems. By operating the cavity in resonance with both the excitation wavelength and Raman wavelength, we attain very high signals, from trace gas components. Our previous results showed estimated sensitivities to CO\(_2\) isotopologues in the low ppm to high ppb range. However, significant challenges to measurement reproducibility prevented proper quantification of the method. New results highlight the instrumental variables that affect the microcavity performance and have led to a new sensor design that improves the sensor stability, and enables quantitative measurement and rapid tuning.

**Keywords:** Forensics, Infrared and Raman, Nuclear Analytical Applications, Trace Analysis  
**Application Code:** Homeland Security/Forensics  
**Methodology Code:** Vibrational Spectroscopy
The Color of STEM; One on One

STEM (Science, Technology, Engineering, Mathematics) is the latest in a variety of education methods. Most of the STEM environments are designed for K-12 students in a group setting. As a pilot study with the Bay City Academy, QuadSil was paired with one student from the Second Grade (carried over to the third grade) to do real research with QuadSil chemists in a business laboratory setting, one day a week during the school year. The student was tested before and after the year(s) to determine comprehension level and improvement in critical thinking skills. The student will present his research effort accompanied by a review of the student’s comprehension level and testing results.

In order to expand Isaac’s education at the practical level, he chose a project on sucrose extraction of sugar beets. This project included starting with raw sugar beets through the entire process of sucrose production and beyond. This included his setting up a project (problem identification, potential solutions, current technology, application and execution). The entire process involved STEM procedures. This included critical thinking, process application, mass balance, extraction, separation, testing and presentation.

Keywords: Chemical, Education, Laboratory
Application Code: Agriculture
Methodology Code: Education/Teaching
In this study, a 3D surface enhanced Raman scattering (SERS) substrate is fabricated, which is composed of electrospun poly(caprolactone) (PCL) fibers and silver coated gold nanorods (Ag/AuNRs). Driven by electrostatic interactions, poly(diallyldimethylammonium chloride) (PDADMAC) and poly(sodium 4-styrene sulfonate) (PSS) are alternately layer-by-layer assembled onto the fiber surface followed by the deposition of a layer of Ag/AuNRs. The 3D architecture of the substrate provides a large surface area for a uniform immobilization of Ag/AuNRs. This fabrication strategy could provide a high magnification and reproducibility of the normal Raman signals, thus its application as an environmental sensor for heavy metal ions detection has been developed as well.

Keywords: Environmental Analysis, Sensors, Surface Enhanced Raman Spectroscopy
Application Code: Environmental
Methodology Code: Vibrational Spectroscopy
The Detection of Catalytic Intermediates in [2+2+2] Cycloaddition Reactions by NMR and ESI-MS

Our research group specialises in the design and optimization of processes to obtain polycyclic compounds using transition-metal complexes as catalysts. Specifically, we have explored [2+2+2] cycloaddition reactions using rhodium-based catalysts and involving allenes as unsaturations. Such processes have been studied from both experimental and theoretical points of view in order to rationalize the relative reactivity of allenes with respect to other unsaturations present in the system.

Mass spectrometry and nuclear magnetic resonance constitute two powerful tools to identify metastable intermediates and propose the most likely reaction pathways through which the system may evolve. In this presentation, we present the main results that we have obtained and the conclusions that we have drawn from their use.

In the first instance, ESI-MS allowed us to record m/z values of some intermediates in a reaction catalyzed by [RhCl(CO)2]2. Complemented with the isotopic pattern, the signals observed revealed to us that the catalytic cycle actually involved the gradual loss of two carbonyl ligands once the catalytic species had been coordinated to the substrate.

On the other hand, the recording of NMR spectra of the crude of a [RhCl(PPh3)3]-catalyzed process allows us confirm the identity of the first coordination intermediate involved, as well as the full loss of the triphenylphosphine ligands once the substrate enters the catalytic cycle. To do this, it was necessary to perform mono- and bidimensional NMR experiments such as 1H, 13C and 31P-NMR, as well as 1H-1H COSY, 1H-13C HSQC, 1H-13C/1H-31P HMBC and TOCSY experiments.

Keywords: Identification, Mass Spectrometry, Molecular Spectroscopy, NMR
Application Code: Other
Methodology Code: Molecular Spectroscopy
Universal Detection of Body Fluid Traces In Situ with Raman Hyperspectroscopy for Forensic Purposes

Towards closing the technological gaps in forensic science, Raman spectroscopy has been a boon to the field. One area that this technique shows exceptional promise is in body fluid identification and characterization, but substrate interference remains a major impediment to its practical implementation. Here, we present an approach for the universal detection of body fluids regardless of the substrate. This approach, which is based on Raman hyperspectroscopy and Multivariate Curve Resolution (MCR), was applied to datasets representing simulated semen evidence. In every instance, the signal of the fluid was extracted and matched to a reference of semen. This approach has immediate application for body fluid detection and allows for a universal, automatic, nondestructive, on-field method for confirmatory identification of body fluid traces at a crime scene. This approach is applicable where an analyte is either a minor contributor or spatially distributed on a strongly interfering substrate.

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 ability to identify body fluid traces at crime scenes, and preserve any DNA present, is critically important in forensic science. Identification can be difficult because many of the current techniques are specific to one body fluid, and typical biochemical methods are destructive—preventing any further analysis. To develop a universal, confirmatory, nondestructive, approach that can be used to differentiate and identify body fluids, we combined the specificity of Raman spectroscopy with the analytical power of statistical modeling. Raman spectra were collected from 75 body fluid samples, including peripheral blood, saliva, semen, sweat, and vaginal fluid. After preprocessing, samples were split into calibration and validation datasets. Several chemometric analysis techniques were trained and tested to find the best model. Combining classification modeling with variable selection resulted in a single, robust, technique. This enhanced model accurately predicted the identity of 99.9% of the spectra from the calibration dataset, after cross-validation. More importantly, it correctly predicted the identity of 100% of the spectra in the external validation dataset. All five body fluids were successfully discriminated by coupling Raman spectroscopy and chemometrics. This technique is both reliable and nondestructive, offering substantial advantages over the current techniques used to identify body fluids.

This project was supported by Awards No. 2014-DN-BX-K551 and NIJ-2015-R2-CX-0019, 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: Biological Samples, Chemometrics, Forensics, Raman Spectroscopy

Application Code: Other

Methodology Code: Chemometrics
A selected ion flow-drift tube mass spectrometric analytical technique, SIFDT-MS, extends SIFT-MS by the inclusion of a static but variable $[E/N]$ field along the axis of the flow tube reactor in which the ion-molecule chemistry occurs. These techniques are useful for quantitative analyses of vapours present for example in environmental air, food flavour, exhaled breath, urine or cell culture headspace. The ion axial speed is increased in proportion to the reduced field strength $[E/N]$ ($N$ is the carrier gas number density) and the residence/reaction time $[t]$, which is measured by Hadamard transform multiplexing, is correspondingly reduced as well. The essential features of SIFDT-MS and SIFT-MS are compared. The resistive glass drift tube is demonstrated to be suitable for SIFDT experiments. The Hadamard transformation can be used to routinely determine reagent ion residence time in the flow-drift tube and also to observe differences in arrival times for different product ions. Two-dimensional data combining arrival time and mass spectra can be obtained. The SIFDT-MS technique can be implemented in a miniature and low-cost instrument and two- or three-dimensional data can be obtained (product ion count rates as functions of $[m/z]$, $[t]$ and $[E/N]$) using the Hadamard transformation. The main features of the technique are compared and contrasted with SIFT-MS and PTR-MS and the latest results are discussed.
Metabolic Analysis of Specific \textit{Lymnaea Stagnalis} Neurons by Capillary Microsampling and Mass Spectrometry with Ion Mobility Separation

The pond snail \textit{Lymnaea stagnalis} (L. stagnalis) is widely used as a simple model organism for neuroscience. Specific types of neurons in the interconnected ganglia of the [i]L. stagnalis[/i] central nervous system (CNS) form neuronal networks responsible for distinct functions that regulate snail behaviors. To understand the correlation between the metabolic makeup and the function of specific neurons, their molecular composition has to be studied on a single cell level. Here, we apply capillary microsampling electrospray ionization (ESI) mass spectrometry (MS) with ion mobility separation (IMS) for the metabolic analysis of specific neurons with known functions in the \textit{L. stagnalis} CNS. The central ring ganglia were removed from an adult snail and kept alive in saline environment. Neurons were identified and sampled by a sharp capillary under an upright microscope. Cell contents were extracted into the capillary that was then used as a nanospray emitter. Metabolite, lipid and peptide ions from single neurons of the visceral ganglion were separated by IMS and analyzed by MS. Molecular identification was based on the combination of accurate masses, collision cross sections (CCSs), and tandem MS. Eventually, 30 metabolites, including AMP, ADP, ATP, GMP, UMP, UDP, glutamine and glutathione, and 4 lipids, including PC(16:1/16:1) and PC(16:0/16:1), were identified out of the ~200 detected ion types. Five peptide ions were also detected in the 1238.0 to 2020.2 mass range. Metabolite compositions were determined for several neurons, including right pedal dorsal one (RPeD1), part of the respiratory pattern generator network, and responsible for learning and memory. The measure characterizing cellular energy content, adenylate energy charge (AEC), for the studied neurons was AEC = 0.78±0.07. Ongoing experiments are aimed to identify the metabolic differences between neurons of specific types and functions.

Keywords: Electrospray, Mass Spectrometry, Metabolomics, Metabonomics

Application Code: Neurochemistry

Methodology Code: Mass Spectrometry
We have developed a compact, continuous-flow microfluidic chip-based chemiluminescence detection system for determination of chromium (III) and chromium (VI) in water. Cr(III) is determined by measuring Cr(III)-catalyzed light emission from luminol (5-amino-2,3-dihydro-1,4-phthalizinedione) oxidation by hydrogen peroxide in basic solutions, and Cr(VI) is determined from the difference between Cr(III) and total Cr concentrations. The total Cr concentration is measured after complete reduction of Cr(VI) to Cr(III) under acidic conditions. Light-shielding black poly(dimethylsiloxane) serves as the substrate of the microfluidic chip that is mounted on the top of a small photomultiplier tube module. The wide linear ranges and the low detection limits are obtained for Cr(III) and Cr(VI): up to 1000 μg/L and 0.13 μg/L, and up to 1000 μg/L and 0.33 μg/L, respectively.
Multifunctional chemical analysis (MCA) systems provide a viable alternative for large scale instruction while supporting a hands-on approach to more advanced instrumentation. These systems are robust and typically use student stations connected to a remote central computer for data collection, minimizing the need for computers at every student workspace. MCA networks offer multiple measurement capabilities, including temperature, potential, drop counting titration, and spectrophotometry. We have built a LED fluorimeter using Lego blocks to provide the ninety degree alignment of the UV LED source (370 nm maximum intensity) and the photodiode. A current-to-voltage operational amplifier circuit permits the photodiode signal to be read by the MCA station. Potential change of the LED source is an advantage of this design. A comparison of this instrument with a commercial LED fluorimeter is understudy. Both instruments are capable of the determination of quinine, the standard teaching experiment, from about 1-20 ppm. The use of a LED fluorimeter for the determination of dansylated amino acids represents an unusual teaching experiment. Arginine as a capsule and lysine as a tablet, both at the 500 mg level, can be found as over-the-counter supplements. Arginine is thought to alleviate some heart and blood vessel conditions while lysine can help prevent cold sores. The commercial fluorimeter generated linear calibration curves for dansyl-lysine from 20-50 ppm and dansyl-arginine from 40-100 ppm. Non-linearity is noted at lower concentrations. Relative error for the determination of these amino acids in supplements averaged 5%.

Keywords: Amino Acids, Fluorescence, Teaching/Education
Application Code: Pharmaceutical
Methodology Code: Education/Teaching
Alcohols are often present in foods and other biological media including exhaled breath, urine and cell culture head-space. Their analysis by chemical ionisation techniques such as SIFT-MS, PTR-MS and SIFDT-MS relies on the ion chemistry initiated by the reactions of the reagent ions H\(_3\)O\(^+\) and NO\(^+\) with alcohol molecules in the presence of water molecules. Kinetics of such reactions needs to be understood and quantitatively described to facilitate reliable quantitative calculation of absolute concentrations of alcohols in humid air samples. The reactions of H\(_3\)O\(^+\) and NO\(^+\) ions have been studied with the primary alcohol molecules in SIFDT analyses (2 mbar He; 0.08 mbar air sample; 300 K; reduced field strength \([i]E/N[i]\) up to 28 Td) and over a range of sample gas humidity up to 5.5%. The H\(_3\)O\(^+\) reactions leads to the formation of protonated alcohol molecules MH\(^+\) and (MH\(^+\)H\(_2\)O) fragments. The NO\(^+\) reactions result in the (M-H)\(^+\) product ions. These primary product ions hydrate to MH\(^+\)(H\(_2\)O)\(_{1,2,3}\) and (M-H)\(^+\)(H\(_2\)O)\(_{1,2,3}\). The variation of the percentages of the hydrated product ions with E/N and with the air sample humidity is studied. The experimental results are compared with a reaction system kinetics model based on numerical solution of a complete set of differential equations describing the ion chemistry. The data resulting from this study including the secondary hydrated ion product distributions will facilitate analyses of alcohol vapours in various media.

**Keywords:** Air, Chemical Ionization MS, Environmental/Air, Volatile Organic Compounds
A critical step in the Counter-IED effort is identification of the most commonly used precursors of improvised explosives devices (IEDs)/homemade explosives (HMEs) in the field. Despite many laws and international regulations in place, monitoring and stopping the flow of restricted materials is a considerable challenge, especially in regions with porous borders or low resources. Most field kits rely on colorimetric liquid chemistry: reactive chemical reagents, such as strong acids and flammable organic solvents, are encapsulated in glass ampules/plastic cases or in dropper bottles, and have to be mixed or applied in a specific timed sequence to obtain results.

A paper-based detection device will be presented for the on-site identification of compounds used to make home-made explosives; it only requires a few drops of water for wetting the suspicious powder. Therefore, the use of hazardous liquids added to potentially reactive unknown samples is avoided. Based on the specific chemical reactions, the color pattern on the device identifies within one minute up to five separate threats (ammonium nitrate, or urea nitrate, or perchlorates, or black powder, or chlorates and organic peroxides, such as TATP and HMTD) using just one sample. Performance of test strips kept at 50° C (122° F) and 40° C (104° F) will be compared to that of test strips kept at room temperature. In addition, a way of analyzing explosive compounds weaponized with dark powders, e.g., flash powder, mixes of chlorate and aluminum powder will be discussed, since this is a major limitation for the commercially available products.

Keywords: Detection, Forensics, Forensic Chemistry, Identification
Unattended Reaction Monitoring Using Automated Microfluidics Sampler and On-line Liquid Chromatography

In-process sampling and analysis is an important aspect of monitoring a chemical process, both in development and during commercial manufacturing. In pharmaceutical process development, the technology of choice for a substantial portion of this analysis is high-performance liquid chromatography (HPLC). Traditionally, the sample extraction and preparation for reaction characterization have been performed manually. This is often very time consuming, laborious, and not practical for a long process. Depending on the complexity of the sample preparation, there can be variability introduced by different analysts, and in some cases the integrity of the sample can be compromised during handling. While there are commercial instruments available for on-line monitoring with HPLC, they lack capabilities in many key areas such as integration of the sampling and analysis, afford limited flexibility in sample preparation, provide no option for workflow customizability, or offer a limited number of unit operations available for sample processing. This work describes a fully automated sample extraction and manipulation system that is controlled using an intuitive Microsoft Excel based control interface. With integration of on-line HPLC, this system provides for unattended reaction monitoring that allows flexible unit operations and workflow customization. In addition, the system is capable of performing complex operations for on-line sample preparation. The automated system is shown to offer advantages over manual method in key areas while providing consistent and reproducible in-process data.

Keywords: On-line, Process Analytical Chemistry, Sample Handling/Automation, Sample Preparation
Application Code: Process Analytical Chemistry
Methodology Code: Process Analytical Techniques
Vapor Modified Transformation of Gas Ions of Small Molecules from a Range of Proton Affinities in Tandem Differential Mobility Spectrometry: Control of Quantitative Response Using Water Vapor

Tandem differential mobility spectrometry (DMS) is an emerging method of ion mobility spectrometry in which gas ions derived from a substance are characterized and detected using two sequential analyzers (stages) where ions may be processed using distinctive field dependent mobility coefficients. An advantage of this instrument design is the concept of dual stage ion filtering where the characteristic dispersion behavior of an ion is exploited for high specificity of detection using combinations of the pair: separation voltage and compensation voltage. Another advantage of a tandem DMS instrument is that ion modification using small neutral vapors to control and improve separation of ion peaks is separated from ion formation, which may occur in gas atmospheres of sample only. In these studies, measurements by tandem DMS of 20 compounds from 5 chemical families were made using water as the vapor modifier. The influence of moisture in altering compensation voltages was determined and the consequences of increased moisture levels on quantitative response were determined experimentally. Findings are supported by computational modeling of the possible reactions. Response was mapped across a reasonably broad range of proton affinities of analytes.

Keywords: Gas, Quantitative, Spectrometer, Water
Application Code: Other
Methodology Code: Chemical Methods
Laser Induced Breakdown Spectrometry (LIBS) has been considered an interesting technique in chemical analysis. Despite all advantages, quantitative analysis in complex matrix is not an easy task and has been considered the "Achilles heel" of this technique. This fact is due to the complex nature of laser-sample interaction and the difficulty in finding appropriate material to do method calibration. This work deals with methods development for direct quantitative measurement of Fe, K, Mg, Mn, Na and Zn in Pinus sp. wood samples. Three strategies of calibration were investigated: (1) use of wood species containing different mass fraction of analytes; (2) analyte addition calibration curve by adding increasing concentrations of analytes in Pinus sp. sample and (3) matrix match calibration curve by adding increasing concentrations of analytes to cellulose and wood species (Pinus sp., Cedrus sp., Aspidosperma sp., Eucalyptus sp.). The reference values for all samples and standards were obtained after acid decomposition and analysis by ICP OES. In third strategy, principal component analysis (PCA) was used to check the similarities between tested calibration curves. The higher similarity was obtained between Pinus sp. and cellulose calibration curves. This result suggests a similar laser-sample interaction for both matrices. Accuracy of methods showed relative error lower than 20%, indicating the feasibility of direct quantitative measurement these analytes in Pinus sp. samples by LIBS using the evaluated strategies.

We acknowledge FAPESP and CNPq for financial support.

Keywords: Calibration, Elemental Analysis, Laser, Method Development
Application Code: Other
Methodology Code: Atomic Spectroscopy/Elemental Analysis
The influence of the given energy on the mass spectrometric fragmentation of polychlorinated biphenyls (PCBs) was investigated and compared among various chlorine substitution patterns.

A single quadrupole mass spectrometer (MS) was used to obtain the mass spectra of all 209 PCBs in both electron capture negative ionization (ECNI) and electron impact (EI) modes, while a tandem MS (MS/MS) was applied for the further fragmentation study in multiple reaction monitoring (MRM) mode.

The major ions are $M^-$, $Cl^-$ and $[M-\text{n}Cl]^- \text{ in ECNI, and } M^+$ and $[M-\text{n}Cl]^+ \text{ in EI. The ionization in EI mode gave the moleculars more energy and generated higher fragment to molecular ion abundance ratio } ([M-\text{n}Cl]/M^-) \text{ than in EI mode } ([M-\text{n}Cl]/M^+)$. 

In EI-MS, congeners with less 2-substitution chlorine were more robust than others within each homolog, and presented relatively lower $[M-\text{n}Cl]/M^+$ ratio. Among congeners with equal 2-substitution chlorines, less ortho or more para substitution tend to make molecular ions stand rather than decompose. However, congeners with full ortho substitution were more likely to exist as molecular ions than fragments. In ECNI-MS, higher ionization degree was exhibited by higher chlorinated congeners. For congeners with >5 chlorines, the average $[M-\text{n}Cl]/M^-$ ratio increased with higher chlorination.

In EI-MS/MS, the dechlorination of robust molecular ions required higher collision energy, and most product ions were $[M-\text{Cl}]^+$ and $[M-2\text{Cl}]^+$. A logistic regression model was built by linking the abundance of $M^+$ and collision energy. The further dechlorinated $[M-2\text{Cl}]^+$ were suggested to be chosen as the product ions in MRM mode if higher response were needed.

**Keywords:** Gas Chromatography/Mass Spectrometry, Method Development, PCB's, Tandem Mass Spec

**Application Code:** Environmental

**Methodology Code:** Mass Spectrometry
Rapid Analysis of Residual Styrene Monomer and Oligomer in Polystyrene Using Fragmentless Ionization Mass Spectrometry

Polystyrene (PS) is used widely as general-purpose plastic that is mostly reused by recycling. The PS contains styrene monomer and oligomer as impurities, which are formed during a heat-treatment process within a manufacturing process. Generally and usually the impurities are determined with Gas Chromatography, however it takes about 30 min. Ion attachment ionization mass spectrometry that is one of fragmentless ionization mass spectrometry offers rapid analysis of those impurities. In the mass spectrum, one peak indicates one chemical species because no fragmentation exists during the ionization, so that it does NOT require any separation technique. The separation is realized in the mass spectrum. In this study, this technique has been applied for rapid analysis of the impurities in the polystyrene. Samples were heated until about 240 \( ^\circ \text{C} \) in 100 Pa chamber. The impurities were thermally extracted to gaseous phase and detected as (quasi-)molecular ions and then no pyrolysis existed during the heating because the heated temperature was still below the pyrolysis temperature of the polymer. Same samples were heated until about 270 \( ^\circ \text{C} \) as 2nd heating scan and then no more evolved gas species were detected. It means that the thermal extraction as 1st heating was almost perfect. Some examples for usual polystyrene products will be presented.

**Keywords:** Data Analysis, Mass Spectrometry, Materials Characterization, Polymers & Plastics

**Application Code:** Polymers and Plastics

**Methodology Code:** Mass Spectrometry
Bimetallic strips are widely used to convert temperature changes into mechanical displacements. Two metals with different thermal expansion coefficients are fused together and, when being heated, bent in the direction of the metal with the smaller coefficient. Inspired by this principle, we made bipolymer strips with two polymers capable of absorbing organic vapors. When exposing the bipolymer strips to organic vapors, the two polymers swell to different degrees because they absorb different amounts of the vapors. Different polymers such as polystyrene, polybutyl methacrylate, polyvinyl acetate, poly(4-vinylpyridine), polyvinylpyrridone were investigated for sensing acetone, toluene, formaldehyde, benzene, etc. The vapor response was reversible. The morphology of bipolymer strips were determined using atomic force microscopy. The bending of the strips under exposure to different organic vapors as a function of time was visualized with a video camera. An intended application of the bipolymer strips is in “electronic nose” systems.
Recent advances in high-speed and efficient separations, such as the use of ultra-high pressure liquid chromatography (UHPLC), have significantly improved the throughput of sample analysis. However, identification and precise quantitation of co-eluted peaks are still one of the greatest challenges for the case difficult to chromatographically separate peaks due to the similarity in their separation characteristics.

Peak deconvolution algorithm has been developed for extracting target peaks from unseparated peaks by analyzing photodiode array (PDA) detector data using the chemometrics multivariate curve resolution alternating least squares (MCR-ALS) technique. This algorithm can separate peaks for multiple components in absorption spectra and chromatograms by simply specifying the wavelength and time ranges.

The integrated deconvolution function in a Chromatography Data System (CDS) can be used to identify spectra and quantitate peaks after separation of individual components, even for difficult-to-separate peaks for which a standard sample cannot be prepared. Furthermore, because this algorithm separates peaks based only on differences in spectral shape, it can also be used to separate and quantitate peaks for co-eluted isomers having exactly the same molecular weight. This report explains the principle used by the MCR-ALS technique to separate peaks, describes an example of using the newly developed algorithm to analyze a sample with isomers of three components, and evaluates the spectral identification and quantitation performance.

Keywords: Chemometrics, HPLC, Liquid Chromatography, Pharmaceutical
Application Code: Pharmaceutical
Methodology Code: Chemometrics
Ten pharmaceutical active ingredients (PAI) were studied in this investigation which included adenosine, Clonidine, Sumatriptan Succenate, Ciprofloxacin HCl, Levofloxacin, Fluconazole, Ketrolace thrumethamine, Pantoprazole sodium and Triprolidine HCl. Most of these PAIs are used as antibiotics and relievers, and they are also used to treat different kinds of diseases such as constant and recurrent migraines. A reversed-phase liquid chromatography has been developed for separation of a mixture of these drugs. Agilent 1100 series High Performance Liquid Chromatography system with Diode Array Detector were used with Thermo BDS Hypersil C18 (250 X 4.6mm, 5 µm) column at a flow rate of 1.00 ml/min. The chromatographic conditions involved a detection wavelength at 270 nm, and mobile phase contains solvent A (25mM potassium phosphate monobasic buffer pH 2.9) and solvent B (acetonitrile). A linear gradient elution was chosen as the elution mode with 5-95 % gradient range. DryLab® software was used to determine the optimum separation conditions. One parameter modeling was tested to determine optimum gradient time, pH, and solvent type, while two parameters modeling was used to determine the optimum gradient time and ternary solvent. In addition, three parameters modeling was also studied to determine the optimum gradient time, temperature and ternary solvent. The develop method was validated and was considered robust.

Acknowledgment: Special thanks to Dr. Imre-Molnar of the Molnar-Institute for applied chromatography for his generous donation of Dry Lab Software.

Keywords: Method Development, Pharmaceutical, Separation Sciences, Validation
Application Code: Pharmaceutical
Methodology Code: Liquid Chromatography
Over the past several years solid-core particles have been gaining increased popularity due to the efficiency benefits that can be achieved. This presentation will focus on additional benefits of solid-core columns, including increased efficiency, decreased sample run time, scalability between multiple particle sizes and selectivity differences between different bonded phases.

Multiple applications of solid-core particles will be presented. Increased peak capacity for the analysis of antihistamines, modernizing of the USP assay method for dofetilide in order to decrease sample run time without sacrificing efficiency, and a selectivity study using various solid-core bonded phases will be shown. The work presented will show the benefits of solid-core particles and how LC analysts in various fields can best utilize them.
The current work on Linagliptin in its bulk drugs, included development of a stability-indicating reverse-phased liquid chromatographic method and its validation for the estimation related substances. Linagliptin related substance was successfully isolated on a semi-preparative high performance liquid chromatography (HPLC) and characterized with the help of high-resolution mass spectrometry, 1H-NMR spectroscopy. Possible structure of the related substance was proposed with the help of mass measurement and NMR spectroscopy. The chromatographic separations were accomplished on Waters Cosmosil C18 column (250 mm x 4.6 mm; 5 µm) using 10mM ammonium acetate and methanol as a mobile phase with gradient elution at 1.0 ml/min flow rate, and eluents were detected using photo diode array detector at 243 nm wavelength. The method was validated with respect to accuracy, precision, linearity, robustness, and limits of detection and quantification as per International Conference on Harmonization (ICH) guidelines.
Pharmaceutical - LC, MS, GC, and LC/MS

Extraction and Analysis of Albendazole from Suspect Pharmaceuticals

According to the World Health Organization, counterfeit pharmaceutical drugs pose a serious and increasingly widespread health risk for people living in developing countries where access to quality medicines are highly limited. The objective of this research was to verify the chemical integrity of Nepali pill samples through colorimetric and quantitative analysis with use of paper-based analytical devices (PADs) and Ultra High Performance Liquid Chromatography, using PDA and MS detectors. In the summer of 2016, samples of albendazole, a deworming agent, was collected from different pharmacies in the Southern, Northern, South Eastern, and Kathmandu regions of Nepal. A UPLC chromatography method was developed to separate and detect albendazole as well as some potential substituted APIs. Sample preparation methods were optimized using a microwave assisted extraction on standard medicines obtained in the United States. Using this method, total ion chromatography (TIC-MS) was used to qualitatively determine drug content and to quantitatively determine the concentration of 133 albendazole samples. The instrumental data validated the results of the pills run on colorimetric PADs. Results showed an agreement between instrumental methods and PADs that albendazole was detected in their appropriate pills as well as no substituted APIs for all Nepali albendazole samples. Quantitative results suggest that some pills may be under dosed and further work will be done on those samples.

Keywords: Liquid Chromatography/Mass Spectroscopy, Pharmaceutical
Application Code: Pharmaceutical
Methodology Code: Liquid Chromatography/Mass Spectrometry
Cost-effective use of chromatographic techniques are effective in obtaining significant quantities of enantiomerically pure drugs and/or drug intermediates. Among several options, closed-loop recycling is thought to be preferable to conventional single column HPLC when resolution is poor. Simulated moving bed (SMB) is further recognized as a powerful tool for industrial-scale separation of enantiomers because of its feature for continuous production and solvent saving.

We compared the productivity and solvent consumption of three separation techniques using: 1) a batch semi-preparative HPLC, 2) a lab-scale recycle HPLC system and 3) a lab-scale SMB. A model case was built with our polysaccharide chiral stationary phase (Cellulose-coated type chiral column) and an authentic sample (Flurbiprofen). It was found that the productivity was 1.5 and 2.5 times greater for continuous methods, and 85% and 97% of solvent was saved on recycle HPLC and SMB respectively when compared with the single column HPLC batch approach. We also estimated the purification cost based on the experimental results. When considering standard equipment depreciation, labouring and solvent cost, this model case shows that the recycle HPLC will provide good cost-effectiveness up to 500g-scale purification, and the SMB can drastically reduce the purification cost over 500g or greater scale.

Keywords: Chiral, Liquid Chromatography, Pharmaceutical, Prep Chromatography
Application Code: Pharmaceutical
Methodology Code: Liquid Chromatography
The role of chiral separation is especially important in the pharmaceutical industry and the demand for isolating enantiomers is a key technology. To provide the required amount of enantiomers rapidly and ensure their optical purity, novel chiral stationary phases (CSP’s) and a rational scale-up process are very important.

Recently YMC developed 5 kinds of CSP’s consisting of polysaccharides derivatives coated or immobilized on 3, 5, 10, 20 [micro]m silica particles. They show excellent chiral separations for a wide range of racemic compounds, and better value than competitive products. These CSP’s give preferable peak shapes without peak tailing on ionic compounds. In these cases, 2-10 times higher loading ability can be expected compared with conventional CSP’s.

Furthermore, these new CSP’s are designed to have the same separation characteristics across particle sizes. We selected a Cellulose-immobilized type chiral column and Propranolol for a test case of the scale-up study. The separation could be linearly scaled-up from analytical 4.6 mm I.D. column to semi-preparative 20 mm I.D. column and also to a preparative 50 mm I.D. dynamic axial compression column packed with bulk packing material, resulting in robustness and efficiency for the scale-up purification.

Keywords: Chiral, Liquid Chromatography, Pharmaceutical, Prep Chromatography
Application Code: Pharmaceutical
Methodology Code: Liquid Chromatography
Abstract Text

When using UV detector for quantitation, the rule of thumb is to select a wavelength where the maximum UV absorbance occurs. The Beer-Lambert Law, however, does not restrict itself to the maximum UV. Any point on the curve of a UV spectrum, by theory, should follow the law and can be used for quantitative analysis. Therefore, in some cases, we “take the advantage” of that fact and select wavelengths other than the maximum for some very good reasons.

Some over-the-counter drug products contain multiple active pharmaceutical ingredients (APIs) with drastically different potencies. For example, a 325 mg to 500 mg acetaminophen can be in the same product that contains a 5 mg to 10 mg phenylephrine. For the sake of efficiency, analytical scientists tend to analyze the APIs with less sample preparation steps or without having to make multiple injections. To achieve that, analysts are attracted to select a wavelength where the large potency API has a weak UV absorbance while the low potency API has a strong UV absorbance.

This approach can, however, backfire. In this poster, we designed some experiments to illustrate the importance of carefully selecting appropriate UV wavelength for quantitation. To make things complicated, in one example, the use of appropriate wavelength unexpectedly brought some interesting challenges in solution stability. In another example, we have to balance the pros and cons between injecting small volumes of sample versus performing second dilutions.

Keywords: Detection, Liquid Chromatography, Pharmaceutical, Sample Preparation

Application Code: Pharmaceutical

Methodology Code: Liquid Chromatography
Ensuring Quality Data for USP 232 Implementation with ICP-MS

The deadline for implementation of USP 232 for the measurement of elemental impurities as the updated method for USP 231 (an optical comparison method) is January 1st 2018. The drive for this updated method is that impurities in pharmaceutical products are of great concern not only due to the inherent toxicity of certain contaminants, but also due to the adverse effect that contaminants may have on drug stability and shelf-life. This necessitates the monitoring of organic and inorganic impurities throughout the pharmaceutical manufacturing process, from raw ingredients to final products.

ICP-MS is an ideal tool for multi-element determination of the 4 USP Class 1 elements (Cd, Pb, As, Hg) as well as the many specified secondary metals at very low concentrations. The paper describes the applicability of various instrument-based techniques to enable trace elemental analysis in the context of the USP Chapters (232, 233 and 2232), and more specifically how to produce consistent quality data for inspections and proof of compliance using ICP-MS. The complete workflow will be discussed from sample preparation, automated sample introduction, method development and interference free analysis to data reporting. Recent developments with an experimental formulation reference material aimed at assisting with laboratory implementation of USP 232 / 233 will also be discussed.

When considering the changes in regulations defined by USP and other international bodies, ICP-MS represents a future proof investment for pharmaceutical laboratories embarking on elemental impurity analyses. The methods developed using ICP-MS exceed the analytical performance criteria described in USP <233> by a wide margin.

Keywords: Automation, Drugs, Toxicology, Ultratrace Analysis
Application Code: Pharmaceutical
Methodology Code: Mass Spectrometry
According to the World Health Organization, counterfeit pharmaceutical drugs pose a serious and increasingly widespread health risk for people living in developing countries where access to quality medicines are highly limited. The objective of this research was to verify the chemical integrity of Nepali pill samples through colorimetric and quantitative analysis with use of paper-based analytical devices (PADs) and Ultra High Performance Liquid Chromatography, using PDA and MS detectors. In the summer of 2016, samples of ciprofloxacin were collected from different pharmacies in the Southern, Northern, South Eastern, and Kathmandu regions of Nepal. A UPLC chromatography method was developed to separate and detect ciprofloxacin as well as some potential substituted APIs. Sample preparation methods were optimized using a microwave assisted extraction on standard medicines obtained in the United States. Using this method, total ion chromatography (TIC-MS) was used to qualitatively determine drug content and quantitatively determine the concentration of 128 ciprofloxacin samples. The instrumental data validated the results of the pills run on colorimetric PADs. Results showed an agreement between instrumental methods and PADs that ciprofloxacin were detected in their appropriate pills as well as no substituted APIs for all Nepali ciprofloxacin samples. Quantitative results indicate that some samples may be under dosed and further work will be done on these samples.

Keywords: Liquid Chromatography/Mass Spectroscopy, Pharmaceutical
Application Code: Pharmaceutical
Methodology Code: Liquid Chromatography/Mass Spectrometry
### Session Title:
Pharmaceutical - LC, MS, GC, and LC/MS

### Abstract Title:
Analysis of Glucocorticoids by GC-VUV

### Primary Author:
Anumeha P. Muthal
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### Co-Author(s):
Nicholas H. Snow

### Abstract Text:
This study focuses on the performance of a new Vacuum Ultra-violet detector (VUV) for analyzing the glucocorticoids. These are the steroidal hormones commonly used in allergic or inflammatory conditions, however these are frequently used in the adulteration of herbal medicinal products to fasten the healing process. Previously studies have been done using GC-MS/MS(2). This study uses a VUV detector (VGA-100) coupled to a gas chromatograph (GC) can test gas phase absorption in the UV range from 125-240 nm. Glucocorticoids were detected using VUV detector as these class of drugs show absorbance with the limit of detection from 500-1000 ppm of the standard glucocorticoid mix. GC-VUV shows a high potential for gas chromatographic analysis, complimentary to mass spectrometry. This technique can also be extended for the applications in various samples to overcome the shortcomings of the other traditional GC detectors.

### References:
2. Schmidt M. QuEChERs (Quick, Easy, Cheap, Effective, Rugged and Safe) Extraction – Gas Chromatography for the analysis of drugs 2015, Dissertations, 102-141.

### Keywords:
- GC, UV-VIS Absorbance/Luminescence

### Application Code:
Pharmaceutical

### Methodology Code:
Gas Chromatography
In recent years, separation of basic compounds using high pH mobile phases has gained considerable interest due to extended retention, excellent peak shapes, and good chromatographic efficiency. The basic rule for elution of compounds in HPLC is to keep the pH of the mobile phase approximately 1 to 2 units higher than the pKa of the analyte of interest; in this way the polar analyte is better retained on the reverse phase column matrix. A significant decrease in sensitivity with electro-spray mass spectrometric detection in positive ion mode (ESI+-MS) is expected under conditions that suppress analyte ionization in solution. We observed that high pH mobile phases do not suppress the ionization of basic compounds in ESI. For example, the response of basic opioids and cannabis in LC-ESI+-MS/MS using 0.1% formic acid in water and methanol was compared to response in 10 mM ammonium bicarbonate buffers (and methanol) at pH 10. Contrary to expectation, high pH mobile phases do not have a negative effect on the response of basic compounds in ESI+. Instead, analyte response and limits of detection are comparable or frequently better in high pH compared to acidic mobile phases.

**Abstract Text**

In recent years, separation of basic compounds using high pH mobile phases has gained considerable interest due to extended retention, excellent peak shapes, and good chromatographic efficiency. The basic rule for elution of compounds in HPLC is to keep the pH of the mobile phase approximately 1 to 2 units higher than the pKa of the analyte of interest; in this way the polar analyte is better retained on the reverse phase column matrix. A significant decrease in sensitivity with electro-spray mass spectrometric detection in positive ion mode (ESI+-MS) is expected under conditions that suppress analyte ionization in solution. We observed that high pH mobile phases do not suppress the ionization of basic compounds in ESI. For example, the response of basic opioids and cannabis in LC-ESI+-MS/MS using 0.1% formic acid in water and methanol was compared to response in 10 mM ammonium bicarbonate buffers (and methanol) at pH 10. Contrary to expectation, high pH mobile phases do not have a negative effect on the response of basic compounds in ESI+. Instead, analyte response and limits of detection are comparable or frequently better in high pH compared to acidic mobile phases.

**Keywords:** Chromatography, Mass Spectrometry, Pharmaceutical, Solvent

**Application Code:** Pharmaceutical

**Methodology Code:** Liquid Chromatography/Mass Spectrometry
Analysis of product aroma via SPME GC/MS is a well-established technique. It can be correlated to aspects of taste perception via expert sensory panels and consumer ranking of products. However, aroma/olfaction alone is not a sufficient measure of the complete consumer experience. This area of applied analysis is ripe for innovation via instrumental and data analysis hyphenation. Drug substances used in OTC (over the counter) cough/cold products are often extremely bitter, low taste detection threshold compounds. As such, a superior analysis platform for prediction of desirable consumer experience needs to be capable of evaluating the aroma (headspace GC/MS) and taste (electronic tongue taste sensor) of products in a combined fashion. We have developed a product analysis platform that demonstrates >90% correlation with consumer-ranked taste attributes. Our efforts to combine GC/MS and taste sensor data into a consumer-predictive model is described herein. Appropriate sampling strategies, GC/MS method development, electronic tongue method optimization and data combination approach will be discussed in detail.
To develop an HPLC/UV size-exclusion method (SEC) for the determination of stability of a large protein and a concurrent UV/Vis spectrometer method for rapid determination of protein concentration in aqueous formulations. The selectivity of the SEC method enabled separation of the large protein into multiple peaks of varying molecular weight from the aqueous matrix, as well as, background interference from deionized water. In addition, the UV/Vis spectrometer method enabled quick turn-around of results. Both methods enabled a simple processing procedure to be used.

For the SEC method, an Agilent 1200 HPLC coupled with a UV/Vis detector was used for quantitation. Chromatography was achieved with a Waters Acquity UPLC BEH200 SEC analytical column fitted with Acquity UPLC BEH200 SEC guard column (column temperature of 30°C), utilizing an isocratic elution. Data acquisition and analysis were performed using Dionex Chromeleon® software version 6.8.

For the UV/Vis method, a Molecular Devices SpectraMax M2 set to 280 nm was used for quantitation. Data acquisition and analysis was performed using SoftMax® Pro Enterprise software version 4.8 and in Microsoft Excel, respectively.

With the SEC method, a quadratic-fit with 1/x weighting was demonstrated from 0.100 to 10.0 µg/mL. Furthermore, stability of the large protein was demonstrated under multiple storage conditions. The LOQ of the large protein was 0.100 µg/mL. A sample ran on both methods had a 3.2% difference in concentration between the two methods.

A simple and accurate method was developed and validated for the quantitation of a large protein via SEC and UV/vis spectrometry.
Due to emissions in the UVA region of the electromagnetic spectrum, standard fluorescent bulbs may not be suitable for room illumination during manufacture and packaging of pharmaceutical compounds labelled as photosensitive. Typically, the manufacture and packaging of products containing photosensitive compounds requires facilities to be illuminated with sodium vapor lamps. The sodium vapor lamps have a narrow emission band at approximately 580 nm, which make them ideal for reducing the photodegradation risk; however, they are not desirable from an operator comfort perspective. The more recent availability of LED lights for general illumination provided the opportunity to evaluate the impact of these light sources on photodegradation, since they have a spectrum cutoff at approximately 400 nm. The impact of LED lights on photodegradation was studied by comparing the photodegradation behavior of known photosensitive pharmaceutical products under fluorescent and LED lights, at equal irradiances (as measured in Lux). Results indicate that degradation under LED light is significantly less as compared to light emitted by fluorescent bulbs, trending with how the emission spectrum of a light source overlaps with the absorption spectrum of the compounds studied.
The Versatile Use of Portable Instruments

The Role of Spatial Orientation in FDM 3D Printing to Spatial Definition of Printed Slit and Optical Detection Performance

Optical detection is the most common detection mode in flow-based analytical techniques such as liquid chromatography (LC), capillary electrophoresis (CE), and flow injection analysis (FIA). Most commonly it is performed on-capillary/column and with the tubing/capillary dimensions changing between methods, optical detectors have been designed committed to match with specific tubing/capillary dimensions. The general goal of this research is to develop an optical photometric detector with a design universally applicable to a range of capillary/tubing outer diameters with the specific aim in this work is to investigate the role of spatial orientation in fused deposition modelling (FDM) 3D printing to spatial definition of printed slit and the device detection performance.

Our approach makes use of a low cost consumer grade FDM printer to enable a high accessibility and rapid turnaround in the design-3D-printing- testing cycle of complex designs. To make this approach suitable for the fabrication of detector housing for capillaries down to 50 µm i.d., the dimension of the slit is a critical element of the detector housing. The spatial definition for a consumer grade 3D printer in terms of printer resolution, determined by layer thickness and layer orientation, is currently around 50 µm. Here, the real limits of spatial resolution and the impact of the orientation in FDM 3D printing were explored. A 70 µm slit was printed, characterised and tested by FIA and CE with a 500 and 200 µm. i.d. fluorinated ethylene propylene tubing and 100, 75 and 50µm i.d. fused capillary giving satisfactory results.
In this work, the performance of a new commercially available miniaturised 6-port injection valve for capillary high performance liquid chromatography (HPLC) featuring light weight (ca. 30 g) and small size (18.3 mm in diameter, 64.9 mm height) was evaluated. It can be easily integrated in a miniaturised modular medium pressure capillary LC system based on off-the-shelf components assembled on a breadboard of a commercial flexible microfluidic platform [1]. The internal volume of the injection valve was determined as 98 nL, and injection loops made of standard polyimide coated fused silica were used, giving total injection volume from 178 nL to 1.3 µL. With the spring loaded rotor adjusted to yield maximum operating pressure measured at up to 320 bar (4641 psi), no change of the injection performance at different backpressure values could be observed. The analytical performance parameters of the miniaturised injection valve were demonstrated by chromatographic separation of common cations and biogenic amine on a miniaturised portable LC system, showing an excellent reproducibility of peak areas with < 2% relative standard deviation (RSD).


Keywords: Instrumentation, Liquid Chromatography
Application Code: General Interest
Methodology Code: Portable Instruments
The Versatile Use of Portable Instruments

Portable Spectroscopy for Teaching Engaging, Immediate and Interactive Science

A challenge in science education is engaging students with interactive and value driven science and with instant feedback. This project demonstrates how ideal portable elemental analysis technology is for teaching the significance of science in society, rewards of gained scientific knowledge and theoretical-to-applied science, while providing more “aha” moments for teachers.

Some portable XRF analyzers used were preconfigured for point-and-shoot testing of Y/N, P/F or quantification. Others gave control of atmosphere, power, filters and collimators with comprehensive qualitative and quantitative software.

Introductory projects illustrate how point-and-shoot tests on everyday samples like coins, jewelry and toys bring the periodic table to life. Project cases include a simple forensic determination of how Ag% identifies genuine or fake coins, a quick evaluation of how variations of Au% are used for karat ratings in jewelry and the identification of safe toys and trinkets based on regulated levels of Pb in PPM.

Intermediate projects illustrate how comparative spectra can provide information such as whose hair has more Cu, Zn or other metals; which produce contains more elemental nutrients such as Mg, K and Ca; and how Pb energy peaks in various concentrations of water can be plotted into linear calibration curves to determine amounts in local water sources.

Advanced projects illustrate the importance of representative sampling such as the benefits of in-situ mapping of a field for heavy metal “hot spots” and how deep and wide metals migrate in given conditions and how precision varies from fully prepped, bagged and in-situ samples and how important blanks are to prevent cross contamination of results.

Technology projects illustrate photon physics by comparing energy spectra of samples with different atmosphere (air, helium, vacuum), filters, kV-µA settings and collimators; and how density and other elements affect an element’s detection and quantification.

Keywords: Education, Elemental Analysis, Portable Instruments

Application Code: Other

Methodology Code: Portable Instruments
The Versatile Use of Portable Instruments

A Next Level in Taking Your Lab to the Sample - True Mobility in Gas Chromatographic Gas Analysis

Ever run into a situation where speed of analysis and reliable results were asked in remote or difficult to reach locations? Promised portability still requires extra equipment. Besides the analytical instrument, gas and power supply, housing, laptop, communication cables are needed. Imagine having to descent into a coalmine to do some measurements and having to drag all this. Besides the proper physical condition, you should also have knowledge on complicated hardware AND software.

We developed a number of improvements that will make operating in rough conditions easier. First of all, we improved our Micro GC’s field case. We took the industry standard Pelican case (designed for ruggedness protecting your valuable Micro GC under the most severe conditions) and designed the interior in such a way that it allows to hold the Micro GC, batteries and gas cylinders. Furthermore, an LCD screen was added for instrument readings, power and gas levels. Equipped with a trolley function and handle bars, it allows you to reach even the most difficult and remote locations.

Secondly, we made it possible to wirelessly connect to the Micro GC via your phone or tablet. This will eliminate the use of a laptop and cables, reducing your carry-on luggage significantly. The webserver will allow you to do (simple) calibration, start the analysis and view the results. Results can be viewed in full but it is also possible to have it reported in “true/false”. In the same method alarms can be set to further improves the follow up action by the operator.

The poster/oral will present all innovations and performance.

Keywords: Fuels\Energy\Petrochemical, Gas Chromatography, Portable Instruments, Process Monitoring
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Portable Instruments
The Versatile Use of Portable Instruments

Helium discharged photoionization detectors (HDPIDs) have been used in many gas chromatography (GC) systems. However, they are bulky and power intensive and cannot be used in a miniaturized GC for field applications. Here we develop a miniaturized HDPID (micro-HDPID) for μGC system, which offers low power consumption (<400 mW), low helium consumption (5.8 mL/min), rapid response (as fast as flame ionization detector - FID), quick warm-up time (~5 min), an excellent limit of detection (a few pg), a large linear dynamic range (>4 orders of magnitude), and maintenance-free operation. Furthermore, the μHDPID can be driven with a miniaturized (~5 cm × ~2.5 cm × ~2.5 cm), light (22 g), and low cost (~$2) power supply with only 1.5 VDC input. The dependence of its performance on bias voltage, auxiliary helium flow rate, carrier gas flow rate, and temperature was also systematically investigated. Finally, the micro-HDPID was employed to detect permanent gasses and a sub-list of the EPA 8260 standard reagents that include 52 analytes. We will describe the details of fabrication and characterization of the micro-HDPID, and show how it is implemented in GC system for in-situ, real-time, and sensitive gas analysis.

Abstract Text

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Abstract Title
GC Detector

Session Title
The Versatile Use of Portable Instruments

Date: Tuesday, March 07, 2017 - Morning
Time:
Room: Exposition Floor, Aisle 2500-2600

Keywords: Detector, GC, Sensors
Application Code: General Interest
Methodology Code: Gas Chromatography
The Versatile Use of Portable Instruments

Fully Automated Portable Comprehensive 2-Dimensional Gas Chromatography Device

We developed first of its kind a fully automated portable 2-dimensional (2-D) gas chromatography (GC x GC) device, which had the dimension of 60 cm x 50 cm x 10 cm and weight less than 5 kg. The device incorporated a micro-preconcentrator/injectors, commercial columns, micro-Deans switches, micro-thermal injectors, micro-photoionization detectors, data acquisition cards, and power supplies, as well as computer control and user interface. It employed multiple channels (4 channels) in the second dimension ([sup]2[/sup]D) to increase the [sup]2[/sup]D separation time (up to 32 s) and hence [sup]2[/sup]D peak capacity. In addition, a non-destructive flow-through vapor detector was installed at the end of the [sup]1[/sup]D column to monitor the eluent from [sup]1[/sup]D and assist in reconstructing [sup]1[/sup]D elution peaks. With the information obtained jointly from the [sup]1[/sup]D and [sup]2[/sup]D detectors, [sup]1[/sup]D elution peaks could be reconstructed with significantly improved [sup]1[/sup]D resolution. The unique design of the GC x GC device significantly enhances the GC peak capacity while remaining compact in size, thus enabling automated, rapid, and sensitive detection of 50 VOCs within 14 min.

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Keywords: Chromatography, GC
Application Code: Environmental
Methodology Code: Gas Chromatography
Metal sites in biology often exhibit unique spectroscopic features that reflect novel geometric and electronic structures imposed by the protein that are key to reactivity. The Blue copper active site involved in long range, rapid biological electron transfer is a classic example. This talk presents an overview of both traditional and synchrotron based spectroscopic methods and their coupling to electronic structure calculations to understand the unique features of the Blue copper active site and their contributions to function. The relation of this active site to other biological electron transfer sites is further developed.

Keywords: Molecular Spectroscopy, Spectroscopy, Ultra Fast Spectroscopy, UV-VIS Absorbance/Luminescence
Application Code: General Interest
Methodology Code: Biospectroscopy
Synchrotron radiation has enabled major discoveries in structural biology over the past 4 decades. Technology developments and innovations have been essential in making this possible, most notably in source characteristics, robotics, software control and detectors. SSRL’s Structural Biology Program continues to pioneer new developments in technologies, methodologies and applications to enable new discoveries. This talk will highlight both synchrotron light and x-ray laser based developments made over the past few years.

At SSRL, there is a focus on macromolecular crystallography (MC), advanced x-ray spectroscopy and imaging and small angle x-ray scattering (SAXS). Developments and applications on in-vacuum undulator based MC beam lines enabling remote access, study of microcrystals and technology developments related to XFEL MC applications will be highlighted, as well as recent developments in advanced x-ray spectroscopy and SAXS.

The revolutionary new x-ray light source, the LCLS x-ray free electron laser at SLAC, has opened completely new and potentially transformational new lines of research where the unique properties of the LCLS x-rays (fsec pulses, extraordinary peak brightness and coherence) are enabling experiments not feasible with ‘conventional’ synchrotron sources. This talk will briefly introduce the XFELs and LCLS, and provide an overview of recent developments and pioneering research in the area of structural biology.

The operation of SSRL and LCLS at SLAC is supported by the U.S. Department of Energy, Office of Basic Energy Sciences and support in structural biology R&D and operations is provided by DOE Office of Biological Research and the National Institutes of Health Institute of General Medical Sciences (NIGMS).

Keywords: Biomedical, Spectroscopy, X-ray Diffraction
Application Code: Biomedical
Methodology Code: Biospectroscopy
The Pittsburgh Spectroscopy Award

Spectroscopic Insights into the BioSynthesis of Coenzyme B12

Adenosylcobalamin (AdoCbl or coenzyme B12) is a member of the family of corrinoids, which contain a cobalt ion that is ligated equatorially by four nitrogen atoms of a tetrapyrrrole macrocycle called the corrin ring. AdoCbl is used as a cofactor in numerous different enzymes that catalyze the rearrangement of organic substrates. Because AdoCbl-dependent enzymes are susceptible to inactivation via oxidation during turnover, all organisms that employ AdoCbl-dependent enzymes are thought to produce ATP:Co(I)rrinoid adenosyltranferases (ACATs). These enzymes catalyze the replacement of the upper axial ligand of AdoCbl precursors with the 5'-deoxyadenosyl (Ado) group derived from a molecule of ATP. Three non-homologous families of ACATs have been identified based on their quaternary structures and classified according to their distinct biological roles in Salmonella enterica. In collaboration with the Escalante-Semerena group at the University of Georgia, we have used a combination of spectroscopic and computational tools to obtain molecular-level insight into the catalytic cycles employed by these enzymes. Although the three ACAT families employ the same basic strategy for catalyzing the Co–C bond formation step, they display intriguing differences with regards to the mechanism by which they generate the Co(I)Cbl key reaction intermediate.

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Keywords: Biological Samples, Biospectroscopy, Computers, Magnetic Resonance

Application Code: General Interest

Methodology Code: Biospectroscopy
A key to any understanding of the catalytic mechanism of a metalloenzyme is the determination of the composition, structure, bonding, and reactivity of the active site for each of the catalytic intermediates that form along the reaction pathway. For paramagnetic states this information can be obtained through analysis of the electron-nuclear hyperfine and nuclear-electric quadrupole interactions of the metal-ion nuclei themselves, of nuclei that form endogenous metal ligands, as well as of enzyme-bound substrates, inhibitors, and products. These interactions are optimally determined by Electron-NUclear DOuble Resonance (ENDOR) spectroscopy, which most importantly can interrogate freeze-trapped intermediates not amenable to crystallization. Parallel studies of biomimetic inorganic complexes not only illuminate the studies of metalloenzymes, but also probe intrinsically interesting electronic and dynamic properties. Not least, ENDOR can reveal otherwise unknowable details of metal-ion speciation in vivo. This talk will select from among our recent studies, such as the determination of the mechanism by which nitrogenase carries out perhaps the most challenging transformation in biology, the reduction of the N≡N triple bond; the structure and bonding in biomimetic analogs of nitrogenase intermediates; hydride tunneling in enzymes; and the mechanism of radical generation by ‘Radical-SAM’ enzymes.
I will present our work in using super-resolution single-molecule tracking to study transcription regulation by metal-sensing regulators in living bacterial cells. Binding and unbinding of transcription regulators on chromosome constitute a primary mechanism for gene regulation. While many cellular factors are known to regulate their binding, little is known on how cells can modulate their unbinding for regulation. I will describe that two metal-sensing transcription regulators, CueR and ZntR, show unusual concentration and chromosome-conformation dependent unbinding kinetics in bacterial cells, which provide novel mechanisms for facile switching between transcription activation and deactivation in vivo and in coordinating transcription regulation of resistance genes with the cell cycle. If time allows, I will present unpublished work in using similar imaging approaches to probe how the dynamic assembly of the tripartite metal efflux complex CusABC helps the cell in defending against metal stress.
Analytical chemistry can take you to unexpected places: from the bottom of a quarry, excavating tiny fossils to discover when mammoths and Neanderthals roamed Europe, to a Yorkshire field measuring water chemistry to work out if an archaeological site is under threat. From Great Barrier Reef corals to South African ostrich eggshells, collaborations between chemists, earth scientists and archaeologists push analytical science forward and advance our understanding of earth’s history.

Focusing on chiral amino acid analysis, with some soft-ionisation protein mass spectrometry to shed light on degradation and preservation, we will explore how isolation of a fraction of ‘intra-crystalline’ protein trapped within fossil shells enables us to date material over the last 3 million years, a time period critical for our understanding of both human evolution and climate change.

Keywords: Amino Acids, Chiral, Geochemistry, Liquid Chromatography/Mass Spectroscopy
Application Code: Art/Archeology
Methodology Code: Liquid Chromatography/Mass Spectrometry
Evolutionary Metallomics

Living things require a diverse set of elements from the Periodic Table, beyond the bulk biological elements C, H, N, O, P, and S. These dependencies stem from evolutionary innovations in ancient environments. As chemists figured out the molecular mechanisms that determine these dependencies today – giving rise to the field of metallomics – geobiologists (enabled by advances in mass spectrometry) and evolutionary biologists (enabled by advances in computational methods) turned to the geologic and genomic records to understand how these mechanisms came to be. This presentation will survey the state of this emerging coevolutionary tale and its implications for life’s past and future, and for the prospects of life on worlds beyond our own.

Keywords: Environmental, Metals
Application Code: Genomics, Proteomics and Other 'Omics
Methodology Code: Mass Spectrometry
Development of Targeted Metaproteomic Method for Studies of Ocean Metabolism and Change

The oceans are so large and complex that our current understanding has been limited by an ability to efficiently sample and characterize key processes on a global scale. At the Woods Hole Oceanographic Institution, we have developed an environmental proteomic method called targeted metaproteomics which will be coupled to samples collected by a new autonomous underwater sampling vehicle (the AUV Clio) to allow large scale observations of the oceanic microbial biogeochemical system across oceanic time and space. Because proteins reflect gene products that are synthesized in response to environmental stimuli, these proteomic methods have the capability to characterize nutrient stresses in microbial populations, the concentration of metalloenzymes responsible for key biogeochemical reactions, as well as regulatory responses and sensory systems indicative of ocean chemistry. Examples from the Central Pacific Ocean and related laboratory studies on cultured microorganisms (the picocyanobacterium Prochlorococcus) will be described, including estimates of nutrient stress and co-stress on distinct phytoplankton populations, calculations of the nitrogen stoichiometry required for specific adaptive responses, and observations of a surprisingly broad distribution of specific metalloenzymes in the mesopelagic ocean.

Abstract Text

Keywords: Environmental Analysis, Environmental/Biological Samples, Liquid Chromatography/Mass Spectroscopy
Application Code: Genomics, Proteomics and Other 'Omics
Methodology Code: Liquid Chromatography/Mass Spectrometry
### Session Title
The Royal Society of Chemistry's Joseph Black Award

### Abstract Title
Interrogation of PTMs in C. Reinhardtii via MS-Based Proteomics Approaches

### Primary Author
Leslie Hicks  
University of North Carolina at Chapel Hill

### Abstract Text
The ability to exquisitely differentiate biological molecules dictating metabolism and its underlying biochemistry is a challenging and meaningful endeavor, as it underpins both fundamental biological research and applied bioengineering. With an interest in extending biological frontiers using advanced technologies, my laboratory aims to establish methods, methodologies, and concepts to set the foundation for clever, practical, and meaningful applications of mass spectrometry in addressing and answering important biological questions. The seminar will focus on our progress in the elucidation of post-translational modifications, specifically on developing and implementing methods for the identification and characterization of thiol-based regulatory switches as well as phosphorylation in Chlamydomonas.

### Keywords
- Biological Samples
- Liquid Chromatography/Mass Spectroscopy
- Method Development
- Proteomics

### Application Code
- Genomics, Proteomics and Other 'Omics

### Methodology Code
- Liquid Chromatography/Mass Spectrometry
A robust analysis of intact protein complexes in the gas phase using ion mobility-mass spectrometry (IM-MS) is a longstanding goal for structural biology. Recent advances in the online cleanup of protein complexes for top-down proteomics has primed the field for routine analyses of intact protein complexes via MS, however tools are still lacking to comprehensively describe the sequence of such ions directly, as well as their detailed 3D structures by IM-MS. In this presentation, new IM-MS strategies will be discussed that are aimed at these challenges.

First, while top-down sequencing of proteins offers substantial advantages in the characterization of proteoforms, significant challenges remain in achieving full sequence coverage of large protein assemblies directly by MS, particularly in dissociating peptides from core regions of protein structure. We are developing chemical modifications has the potential to fundamentally improve protein complex collision induced dissociation (CID) experiments by revealing unique peptide and protein fragment ions that cover a greater portion of the protein sequence and more fully reflect the structure of the assembly.

To attain 3D structure information, our group has developed collision induced unfolding (CIU) technology, which is capable of assessing the stability and unfolding pathway for gas-phase proteins and complex from small amounts of sample, without the need for labeling. We have recently demonstrated the potential of this approach for biotherapeutic characterization, as well as worked to identify CIU bands as being related to the native domain structure of the ions that have undergone unfolding in the gas-phase. This presentation will cover our latest CID and CIU data, demonstrating the promise of these combined technologies on IM-MS platforms for future applications in structural proteomics.

Keywords: Electrospray, Mass Spectrometry, Protein, Tandem Mass Spec
Application Code: Bioanalytical
Methodology Code: Mass Spectrometry
Native ion mobility mass spectrometry is an emerging approach for characterizing the stoichiometry, assembly, and shapes of protein complexes in solution. Native ion mobility mass spectrometry is especially useful for investigating proteins and protein complexes that are challenging to characterize using condensed-phase experiments, including those that are heterogeneous, have large mass, and are membrane bound. I will discuss how my lab uses structures for lossless ion manipulation (SLIM) to separate, size select, trap, and activate ions of intact proteins and protein complexes in the gas phase. I will then discuss the implementation and application of these technologies in the context of biophysics and structural biology.

Keywords: Bioanalytical, Instrumentation, Mass Spectrometry, Protein
Application Code: Bioanalytical
Methodology Code: Mass Spectrometry
It is well accepted that a measurement of an ion's mobility through an inert gas can be compared with calculated mobilities for trial geometries to obtain insight about the abundances and overall shapes of ions. In these studies, energy can be added to induce structural transitions and follow changes in the abundances of different conformations that are favored before and after activation. Here, we extend this idea as a means of following structural transitions in solution, examining the well-studied model systems: bradykinin, polyproline and ubiquitin. Our approach is to vary the solution composition or temperature from which ions are electrosprayed. Overall, we conclude that in some cases it appears that the solution phase structures are more or less preserved in the gas phase; in other cases, new structures are favored in the gas phase—but, these can be mapped back in order to obtain insight about populations of states that were favored in solution. In the case of bradykinin, we find evidence for ~10 different solution phase states that vary in abundance as the solution composition is changed. These populations largely arise from variations in the cis- and trans-configurations of three proline residues in the nonapeptide sequence. The polyproline system provides a chance to study such transitions in detail. When in relatively non-polar solvents such as propanol, polyproline forms a compact type PPI helix; when placed in water the polymer undergoes a series of cis-trans interconversions to produce a type PPII helix, in which water molecules intercalate along the peptide backbone, stabilizing a much more extended structure in solution. In the case of ubiquitin, solution composition and temperature studies show evidence for new states. Overall, we find that ESI-IMS-MS techniques appear to provide new information about the step-by-step transitions that connect these different structures—information that currently cannot be obtained by any other existing approach.
A tandem differential mobility spectrometer at ambient pressure can provide enhanced selectivity of response with high speed through dual stage ion filtering. The addition of gas modifiers or chemical reagents between two DMS stages creates further specificity based on the introduction of chemistry into gas ion behavior. Examples include displacement reactions, charge stripping reactions and fragmentation. Technology and practices of tandem DMS will be examined as an analyzer after chromatographic separation or before mass analysis.

Keywords: Gas Chromatography, Mass Spectrometry
Application Code: Clinical/Toxicology
Methodology Code: New Method
Ion mobility spectrometry (IMS) coupled with mass spectrometry (MS) has been successfully applied in the analysis of small molecule, biomolecule, and polymer analytes. Though IMS itself can also be employed in the analysis of the sizes and structures of inorganic (metal and metal oxide) nanomaterials, IMS-MS has not been applied in nanomaterial analysis, primarily because the masses of such materials are typically too large for conventional mass spectrometry. This presentation will provide an overview of recent developments in applying IMS as both a standalone and hyphenated technique the analysis of nanomaterials synthesized via both colloidal and gas phase techniques. Specifically discussed will be (1) non-electrospray based aerosolization and ionization techniques for a variety of nanomaterials (gold nanoparticles and nanorods, iron oxide nanoparticles, and titanium dioxide nanoparticles), (2) atmospheric pressure IMS with both differential mobility analyzers and drift tube ion mobility spectrometers, (3) the coupling of atmospheric pressure IMS systems with single particle sensitive condensation particle counters, (4) the use of IMS analyses to quantitatively determine nanomaterial number concentrations and size distribution functions, (5) the use of IMS to examine the extent of protein binding to nanomaterials, and (6) the coupling of IMS with centrifugal based mass analyzers for true IMS-MS measurement of nanomaterial populations. Overall, results to date suggest IMS has a number of advantages over conventional technologies (i.e. dynamic light scattering) in assessing nanomaterial size and mass distribution functions, as well as in examine nanomaterial surface functionalization.
Characterization of the overall topology and inter-subunit contacts of protein complexes, and their assembly/disassembly and unfolding pathways, is critical because protein complexes regulate key biological processes, including processes important in understanding and controlling disease. Conventional structural biology methods such as X-ray crystallography and nuclear magnetic resonance provide high-resolution information on the structures of protein complexes. However, other emerging biophysical methods that provide lower resolution structural data (e.g. stoichiometry and subunit connectivity) on the structures of the protein complexes are also important. Native mass spectrometry is an approach that provides critical structural information with higher throughput on low sample amounts. The power of native MS increases when coupled to ion mobility (IM-MS), a technique that measures rotationally averaged collisional cross sections and thus direct information on conformational changes, or to high resolution mass spectrometry (HRMS). This presentation illustrates surface-induced dissociation/ion mobility (SID/IM) MS and HRMS (orbitrap and ICR) for characterization of topology, intersubunit connectivity, and other structural features of multimeric protein complexes. Data for a number of protein-partner complexes will be presented, where the partner can be small molecule ligand, protein, DNA, or RNA.

Keywords: Chromatography, Nucleic Acids, Protein, Tandem Mass Spec
Application Code: Bioanalytical
Methodology Code: Mass Spectrometry
Advances in Real-Time Detection of Metal Ions for Bioimaging and Environmental Monitoring

New Targeted Fluorescent Probes for the Study of Intracellular Metal Distribution and Mobilization

Fluorescence microscopy imaging is one of the most effective techniques for the study of accumulation and distribution of intracellular cations such as Na\(^{+}\), K\(^{+}\), Mg\(^{2+}\), Ca\(^{2+}\) and the transition metals. Currently available fluorescent indicators for Mg\(^{2+}\), however, lack in selectivity and spatial resolution, thus hampering the study of compartmentalization and trafficking of this ion in the context of physiological and pathological processes. We have developed a new family of triazole-based ratiometric fluorescent probes for the targeted intracellular detection and quantification of Mg\(^{2+}\) in mitochondria, as well as a new general strategies for the activation of fluorescent indicators ‘on demand’ for ratiometric detection of Mg\(^{2+}\) in various subcellular compartments with superior resolution. Our new methodologies are readily applicable to the detection of other metals and analytes, and promise to provide a more complete picture of intracellular metal trafficking and its significance.

Keywords: Biosensors, Fluorescence, Imaging, Metals

Application Code: Bioanalytical

Methodology Code: Microscopy
Tracking Mobile Zinc in the Brain - New Probes, New Biology

Purpose: The goal of this research is to devise and apply new tools for tracking mobile zinc in the brain. Included are novel fluorescent sensors and fast, specific chelating agents for measuring zinc flux in the hippocampus as well as the auditory, visual, and olfactory sensory systems.

Results and Conclusions: Mobile zinc sensors are described that (i) are based on fluorescein and cyanine platforms as light-emitting units; (ii) employ fluoresceins with acetyl protecting groups to facilitate endosomal release in cells, eliminate fluorescent background, and can be deprotected with turn-on by mobile zinc; (iii) contain pH-insensitive spirobenzopyran units that convert to red-emitting cyanine dyes upon exposure to mobile zinc; and (iv) can be incorporated in peptides for cellular penetration and localization purposes as well as providing ratiometric quantitation. Complementary tools for applying these probes include the use of ZnT3 knockout mice and fast zinc chelators for intercepting transient increases in mobile zinc. With collaborators, these probes have been applied to record (i) single synapse release of vesicular zinc in the hippocampus; (ii) modulation of NMDA and AMPA receptors by synaptic and tonic zinc in a class of inhibitory interneurons in the molecular layer of the dorsal cochlear nucleus that receive glutamatergic input from synaptic zinc-rich parallel fibers; (iii) release of zinc from glomeruli in the olfactory bulb upon electrophysiological stimulation in slices and as delineated in sensory maps produced in live animals following exposure to specific odorants; and (iv) mobile zinc release in the inner plexiform layer of the retina following optic nerve crush, a phenomenon used to guide the application of a fast zinc chelator as a means to promote optic nerve regeneration following crush.

This work was supported by a grant from the National Institute of General Medical Sciences.

Keywords: Biosensors, Luminescence
Application Code: Neurochemistry
Methodology Code: Sensors
The exploration of the brain and its distinctive role in forming the center of consciousness offers a grand challenge for achieving a molecular-level understanding of its unique functions, including learning and memory, as well as senses like sight, smell, and taste. As such, the brain also represents a frontier for developing new therapeutics for aging, stroke, and neurodegenerative diseases. We are developing molecular imaging approaches as a way to identify and study the underlying chemistry that governs brain activity. This talk will present our latest results in the discovery and understanding of transition metals as bona fide signaling elements and their influence on neural circuitry, as well as expansion of this concept of transition metal signaling to other biological systems.
Fluorescent tools have launched biological research into a new realm of understanding of cellular processes and dynamics at the single-cell level. These tools are enabling characterization of stochasticity and heterogeneity exhibited by biological systems, which could not adequately be probed by techniques that rely on bulk analysis of populations of cells. Fluorescent sensors are increasingly providing insight into the “dark matter” of the cellular milieu: small molecules, secondary metabolites, metals, and ions. One of the great promises of such sensors is the ability to quantify cellular signals in precise locations with high temporal resolution. Yet this is coupled with the challenge of how to ensure that sensors are not perturbing the underlying biology and the need to systematically measure hundreds of individual cells over time. This talk will highlight our efforts to develop genetically encoded FRET-based sensors for quantitative mapping of zinc ions in cells. I will discuss approaches for defining whether sensors perturb cellular ions, and the specific challenges associated with quantifying ions in cellular organelles. Finally, I will discuss our efforts at systematic quantitative analysis of long-term imaging of ions during the cell cycle to highlight the need for sophisticated image analysis algorithms.

Keywords: Biosensors, Fluorescence, Metals, Microscopy
Application Code: Bioanalytical
Methodology Code: Microscopy
Selective sensors for metal ions are very useful for on-site and real-time detection in environmental monitoring, medical diagnostics and imaging. Despite a lot of effort, designing selective sensors based on a single class of molecules for a broad range of metal ions remains a significant challenge. Most processes are on a trial and error basis where successes in designing agents for one metal ion can be difficult to translate success in designing agents for other metal ions. To meet these challenges, we have been able to use in vitro selection to obtain DNAzymes, a new class of metalloenzymes that use DNA molecules exclusively for catalysis, and use negative selection strategy to improve the selectivity. By labeling the resulting DNAzymes with fluorophore/quencher, gold nanoparticles, gadolinium or supermagnetic iron oxide nanoparticles, we have developed new classes of fluorescent, colorimetric and MRI agents for metal ions with high sensitivity (down to 14 pM) and selectivity (> 1 million fold selectivity) [1]. These sensors have been applied for imaging metal ions in living cells and in vivo to offer deeper insight into their roles in biology [2]. We also have taken advantages of the wide availability and low cost of glucose meters to detect metal ions [3]. Since in vitro selection can be used to obtain DNAzymes selective for almost any metal ion, the methods describe here can be applied for developing sensors for many metal ions in environmental monitoring, medical diagnostics and imaging.


Keywords: Biomedical, Biosensors, Environmental Analysis, Imaging
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 (OIFS) maintains a program of external funding for research and development in the forensic sciences. This program is a leading federal funder in this mission space, and the portfolio spans a broad range, from fundamental research with the potential for application to forensic science, to the development of prototype devices, to the validation of novel instruments and methods. Since 2009, NIJ has funded over $179M of external R&D to support the advancement of accuracy, validity and efficiency in the forensic sciences.

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 drives 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 community 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 fiscal year 2017 [u]Research and Development in Forensic Science for Criminal Justice Purposes[/u] solicitation which will accept applications through February 28, 2017 (more details at http://nij.gov/funding/Documents/solicitations/NIJ-2017-11080.pdf) and relevant examples of past funded projects in the areas of Crime Scene Analysis and Forensic Biology.

Keywords: Forensics, Forensic Chemistry
Application Code: Other
Methodology Code: Chemical Methods
Sampling and analysis of the volatiles emanating from forensic specimens provides unique and important information that may aid in the location and discrimination of items of forensic interest. Non-contact sampling of forensic specimens in the field preserves the evidence and can provide for rapid onsite detection as well as preservation of the volatile organic compounds (VOCs) for repetitive and/or complementary laboratory analysis. A variety of sampling and analysis techniques have been developed that have increasingly improved detection limits and number of headspace compounds detected from and increased array of specimens. This presentation describes innovative uses of headspace sampling and analysis and the discriminating power of the resulting data including the use of dogs and sensors. The VOC profiles of forensic specimens including drugs, explosives and human scent have been analyzed to reveal the discriminating power of different techniques and the potential of what is referred to as odor evidence. The increased ability to detect and analyze volatile chemicals associated with forensic materials increases the amount and value is of evidential materials available to forensic scientists and is often crucial to locating and associating crime scent evidence to a suspect(s). Case studies will be shown where odor evidence has been useful to solve crimes and how this form of evidence has been declared reliable in a variety of courts of law including the US Supreme Court.

Keywords:  Biosensors, Forensics, GC-MS, SPME
Application Code:  Homeland Security/Forensics
Methodology Code:  Sampling and Sample Preparation
Macro X-Ray Fluorescence (MA-XRF): A Powerful Tool for the Non-Invasive Detection, Analysis and Imaging of Biological Traces and Gun Shot Residues in Forensic Science

The use of Macroscopic X-ray Fluorescence (MA-XRF) imaging has shown great value in art examinations. MA-XRF allows for the non-invasive elemental analysis and imaging of large surfaces. Because X-rays penetrate the analyzed surface to some extent depth profiling is also feasible enabling art historians, conservators and scientists to visualize and study hidden structures and layers beneath the surface of art objects such as paintings. In this work large area MA-XRF is applied in a forensic context to detect and image biological traces and gun shot residues on entire pieces of clothing. MA-XRF was found to be sufficiently sensitive to detect Fe, K and Cl in human blood, Zn, K and Cl in human sperm, K in saliva and K and Cl in urine and sweat. Furthermore, on the basis of multi-elemental profiles the human biological traces could be distinguished from frequently occurring non-human stains. Detection limits were found to be in low ppm range indicating the added value of MA-XRF to detect biological traces on surfaces that pose problems for conventional optical and fluorescence techniques and IR/NIR spectroscopy. On the basis of elemental differentiation of the impact residues different types of firearm ammunition could be distinguished. Additionally, high resolution imaging of the GSR at the point of impact also provided information about the order and angle of the shots fired. This effectively demonstrates how MA-XRF could aid in the reconstruction of complex shooting incidents. This could even be the case in situations where perpetrators have attempted to conceal evidence at the scene of crime. In a fashion similar to the analysis of hidden features in works of art, MA-XRF was used in this study to detect lead residues from a bullet fired at a plastered wall even if the point of impact was covered with three layers of blue wall paint. This shows the potential of using MA-XRF directly at the scene of crime to detect hidden evidence.

Keywords: Detection, Forensics, Imaging, X-ray Fluorescence
Application Code: Homeland Security/Forensics
Methodology Code: X-ray Techniques
Forensic Analysis in the Lab and Crime Scene

**Collection and Analysis of Breath Components for Marijuana Detection Using Capillary Microextraction of Volatiles (CMV)**

The noninvasive collection of exhaled breath makes for an attractive sample for field detection of drugs from suspected impaired drivers. Previous studies characterizing the breath of individuals who have smoked marijuana used filters as sample collection devices resulting in low collection efficiencies. This study proposes the evaluation of a new technology, capillary microextraction of volatiles (CMV), for its utility in the collection of breath aerosols and volatiles from exhaled breath. The CMV preconcentrates breath components using a mini capillary tube filled with polydimethylsiloxane (PDMS) coated glass filter strips. The CMV offers dynamic sampling of volatile organic compounds (VOCs) with a simple coupling to the inlet of a gas chromatograph (GC) for analysis, avoiding expensive thermal desorption instrumentation needed for bulk sorbent type collection devices. CMV offers a 5,000-fold increase in surface area and an improved collection capacity over the static single solid-phase microextraction (SPME) fiber. In addition, the CMV sampling is dynamic, allowing for a relatively large volume (> 2L) of lung air to be sampled in less than one minute. The collection efficiency and analysis of VOCs associated with normal breath and from VOCs associated with marijuana smoking were studied using a simulation of synthetic breath composed of vapors generated by permeation into a flow of humidified nitrogen. After collection of the synthetic breath onto the CMV, two extraction methods were tested for efficacy in releasing analytes for analysis. Reliable demonstration of the CMV for breath collection would serve as a proof of concept for future applications of the CMV for detection of marijuana smokers’ breath for drug impaired driver management. The portability and sensitivity of the CMV could aid law enforcement agencies during traffic patrols of drug impaired drivers in the future.

**Keywords:** Forensics, Forensic Chemistry, Gas Chromatography/Mass Spectrometry, Sampling

**Application Code:** Homeland Security/Forensics

**Methodology Code:** Sampling and Sample Preparation
Raman microspectroscopy combined with advanced statistics is uniquely suitable for characterizing microheterogeneous samples. Understanding the structure and (bio)chemical composition of samples at the microscopic level is important for many practical applications including material science, pharmaceutical industry, various (bio)analytical purposes, etc. Raman spectroscopy has already found numerous applications in forensic chemistry providing confirmatory identification of analytes. The technique is non-destructive, rapid and requires little or no sample preparation. Furthermore, portable Raman instruments are readily available allowing for crime scene accessibility. We have recently demonstrated that Raman microspectroscopy can be used for the identification of biological stains at a crime scene indicating the type of body fluid. In addition, peripheral and menstrual blood as well as human and animal blood can be differentiated. We will also discuss the application of Raman spectroscopy for determining phenotype characteristics of the donor based on the analysis of body fluid traces.
Biogenic amines (BAs) are key molecules to monitor ageing during storage of protein-rich food. Although the human nose senses high concentrations lower concentrations of BAs may induce adverse effects on health that can potentiate one another in simultaneous presence of various BAs. Inexpensive optical fluorescent sensors or test stripes can avoid time-consuming methods hyphenated to chromatography to quantify all BAs in a sample. We therefore report on the amine-sensitive fluorescent probe (Py-1) to determine BAs in various food samples with various sensory concepts.

For in-field use, we created test spots containing the chromogenic probe Py-1 and a green fluorescent reference dye in a hydrogel matrix. They enable rapid and direct determination of BAs via (1) a color change from blue to red (on dipping into a sample) if the level of BAs reaches harmful concentrations and (2) “fluorescence-photography” with a commercial digital camera after LED-excitation at 505 nm. The ratio of the red (signal) channel and the green (reference) channel is extracted from the RGB-image. The up to 7.5-fold increase of red Py-1 emission at 620 nm to the constant green reference fluorescence at 515 nm yields calibration plots for BAs to be determined at 0.01-10 mM within 15 min.

A reusable sensor microplate with Py-1 embedded in a polymer on the bottom of the wells permits high-throughput screening. The plate enables (1) a semi-quantitative readout of the concentration of BAs by eye-vision, (2) a rapid fluorescence readout with common microplate readers in less than two minutes and (3) a rapid sample preparation. The linear ranges of the calibration plots of six BAs cover the toxicological relevant range of BAs. The ageing of real seafood, meat and cheese samples was monitored via their total content of biogenic amines (TAC). This TAC is related to the concentration of histamine in the sample and was found to agree well with the total levels of BAs found by GC-MS.

Keywords: Analysis, Fluorescence, Food Safety, Sensors
Application Code: Food Safety
Methodology Code: Fluorescence/Luminescence
Food waste is a global social, economic, and political problem and a United Nations study reports that 1.3 billion tons of food are wasted annually (Global Food Waste Report: “Food wastage footprint – impacts on natural resources”, Food and Agriculture Organization of the UN (FAO) 2013). One major challenge in this context is to collect accurate information on food freshness to allow food to be shipped, sold, and consumed before it spoils.

To address this challenge, we are developing chemiresistive sensors that combine a network of single-walled carbon nanotubes (SWCNTs) with selector molecules that interact with analytes of interest. The sensors measure gas markers in the headspace of food items, e.g., ethylene for climacteric fruit or biogenic amines, sulfides, or acetoin for meat, fish, and poultry. We have demonstrated the use of such a sensing system for monitoring the freshness of various meat samples (Fig. 1) using the interaction of biogenic amines with metalloporphyrin-based selector-SWCNT composites.

Due to their low cost and facile readout using resistance measurements, the developed chemiresistors have the potential to be integrated directly into food packaging allowing real-time freshness monitoring as the food travels through the supply chain. In my presentation I will give an overview of SWCNT-based chemiresistive sensors and strategies for evaluating and optimizing their performance using different sensors developed by our groups.
Seafood decomposition occurs when fish and fishery products are subjected to time/temperature abuse. The Federal Food, Drug, and Cosmetic Act (FD&C Act) section 402(a)(1) states that a food product is adulterated “if it bears or contains any poisonous or deleterious substance which may render it injurious to health” and section 402(a)(3) states that a food product is adulterated “if it consists in whole, or in part, of any filthy, putrid, or decomposed substance”. The U.S. Food and Drug Administration (FDA) applies these statutes when seafood products are sampled and analyzed for decomposition. Current guidance considers histamine at levels $\geq 50$ ppm in scombrotoxin-forming fish (e.g., tuna and mahi-mahi) to be evidence of decomposition while histamine at levels $\geq 500$ ppm is considered to be a human health hazard (Compliance Policy Guide Sec. 540.525). Indole at levels $\geq 25$ µg/100g in shrimp is also considered to be evidence of decomposition (Compliance Policy Guide Sec. 540.370). Analytical methods used in FDA laboratories to detect these biogenic amines include ion-exchange chromatography coupled with fluorescence detection for histamine (AOAC 977.13) and high performance liquid chromatography coupled with fluorescence detection for indole (AOAC 981.07). In addition to these analytical methods, organoleptic (sensory) analysis may be conducted to identify decomposed seafood. FDA seafood sensory analysts are highly trained organoleptic specialists who are routinely tested for their sensory acuity in designated seafood product categories. Seafood samples analyzed at FDA may be deemed adulterated when chemical analysis, sensory analysis, or a combination of the two, detects evidence of decomposition and/or a hazard to health. Regulatory applications associated with chemical and sensory analysis of seafood products will be discussed.
Contamination of the food with pathogens not only sicken, but may lead to hospitalization and even death in people with compromised immune systems. Public demand for organic, non-pasteurized food products is inducing pressure on the food industry to provide high quality/safe products, which requires rapid sensors to test food products in situ. While many detection strategies exist, there are few approaches for determining pathogen viability. The objective of this work was to develop a nanobrush material with two distinct features: 1) the ability to selectively capture pathogens, and 2) determination of viability based on the decarboxylation of exogenous amino acids. A mannose binding lectin (concanavalin A, Con A) and the glycoenzyme diamine oxidase (DOx) were assembled in a layer-by-layer approach for developing a multilayer pH-sensitive nanobrush. The outermost layer of the brush was terminated with ConA or a 64mer aptamer to facilitate capture of Escherichia coli O157:H7. Lectin- or aptamer-mediated cell capture was interrogated using electrochemical impedance spectroscopy and cyclic voltammetry. After cell capture, exogenous amino acids were added and metabolism by viable E. coli produced biogenic amines (BA). DC potential amperometry was used to detect BA at +400mV. The optimal lectin and enzyme concentrations for creating the nanobrush layers were determined to be 0.8 mg/mL and 1.0 mg/mL, respectively; with an optimum time of 20 min at room temperature using PBS as a binding buffer. The total assay time for capture/viability was 40 minutes: 10 min for cell capture and 30 min for viability. Ongoing testing aims to optimize the amino acid concentration/type and examine the effect of stimulus response. This new sensor approach can be expanded to target specific foodborne pathogens by altering the outermost capture probe of the nanobrush assembly, enabling rapid determination of food pathogen presence and viability without addition of expensive reagents.

Keywords: Biosensors, Detection, Electrochemistry, Food Safety
Application Code: Food Safety
Methodology Code: Electrochemistry
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. Often, this degradation is caused by liquid-solid and air-water interfaces, and proper formulation design can reduce damage. However, even with optimal formulations, great care is needed to assure proper handling of these products. Unfortunately, handling by end users is often far from optimal. Even routine handling such as IV infusion can cause a huge increase in micro- and nanoparticles, and the latter are not effectively removed from the formulation by in-line filters. Also, in some hospitals IV bags containing product drug solutions are transported by pneumatic tube systems. The mechanical stress caused by this handling can massively increase the subvisible particle levels. This presentation will summarize the causes of protein aggregation and particle formation, rational intervention and control strategies, challenges with particle analyses of protein formulations, and the consequences to patients of administering products with aggregates and particles.

Keywords:   Particle Size and Distribution, Pharmaceutical, Protein, Raman Spectroscopy
Application Code:   Bioanalytical
Methodology Code:   Vibrational Spectroscopy
Many biopharmaceuticals have strong potential as drugs for their high efficacy, and in the biopharmaceutical industry, highly concentrated (>100 mg/mL) liquid formulations are becoming required. At high concentrations, however, the increased viscosity is accompanied by an increased risk of protein aggregation or denaturation. These changes may result in side effects, and therefore the optimization of drug formulations is of great importance. Accordingly, a comprehensive understanding of the interactions or conformation of proteins that could be relevant to oligomerization or aggregation in non-ideal solutions (e.g., highly concentrated solutions) is highly important.

Currently, relatively few techniques are available to directly investigate the conformation in high-concentration solutions. Raman spectroscopy is one of the best methods for analyzing high-concentration solutions. In addition, Raman spectroscopy is a powerful tool for detailed investigation of not only the secondary structure of Amide I band but also the detail behavior of each functional groups such as Tyr and Trp in proteins.

We performed the various application studies and from the experimental results it was confirmed that the secondary structure of the IgG molecule was maintained, although the excluded volume effect worked powerfully in highly concentrated solution. In contrast, it was found that the local environment around the hydrophobic residues such as Tyr and Trp changed slightly. These changes may be induced by the short range attractive interaction, which is specific in a highly concentrated solution and could cause the aggregation. Therefore, the difference between the local environment in a highly concentrated solution and the one in a diluted solution can be observed by using Raman spectroscopy. These results indicate that Raman spectroscopy can be a useful complementary method for the study of highly concentrated solutions.

Keywords: Biopharmaceutical, Protein, Raman Spectroscopy
Application Code: Pharmaceutical
Methodology Code: Vibrational Spectroscopy
Researche on protein hydration structure and aggregate formation show the new era, which has been triggered by development of biotherapeutics. Recent analytical solutions for biopharmaceutical practice have been proposed from these experiences. Here we introduce recent studies on these topics from two viewpoints. First, preferential interactions of additives with proteins are strongly dependent upon the unique surface structures of proteins, which have described marginal effects of arginine on handling proteins. These insights have some clues on the liquid formulation of proteins. Second, detection of protein aggregates, especially for subvisible particles, have been focused on. Immunogenecity of the particles have been suggested, and several methods have been proposed to detect the aggregates. Thermodynamic analyses of biopharmaceutics have provided valuable information with evaluation, validation, and optimization of formulation conditions. Raman spectroscopic analyses have been proved to be powerful for analyses of high-concentration formulation. We discuss recent progresses on the studies from viewpoints of both colloidal and conformational stabilities.
Development of antibody-based drug molecules has improved patient experience during chemotherapy due to the targeted nature of the treatments. Drug treatment efficiencies have greatly improved through antibody conjugation chemistries such as protein PEGylation as these newly designed drugs show improved delivery and higher stability. Unfortunately, relative to their small molecule counterparts, biologics and biosimilars remain difficult to characterize due to their size and complicated dynamic structures. Matrix Assisted Laser Desorption Ionization Time of Flight Mass Spectrometry (MALDI-TOF MS) is the technique of choice for detecting high molecular weight species such as antibodies due to low requirement for sample preparation and the ease of spectral interpretation. The main limitation of time of flight analyzers is that the speed of travel is inversely proportional to the molecular weight and lower travel speed causes less intense collisions with the detectors resulting in decreased sensitivity. In order to circumvent such limitation, a high mass detector (CovalX Zurich, Switzerland) was fitted to a traditional MALDI-TOF MS (Shimadzu Manchester, UK) which resulted in generation of higher sensitivity and higher resolution data up to 2 MegaDaltons (MDa), well beyond the detection limits of a traditional MALDI-TOF system. This presentation focuses on the use of high mass MALDI or MegaTOF (Shimadzu Scientific Instruments Columbia, MD) enabled the successful analysis and enabled a fast determination of the extent of conjugation and aggregation.
A strong demand in drug discovery and development today is to overcome “Big Gaps” encountered by differences in species and races, to accelerate effective developments in cost and time, and to meet medical needs. Moreover, drugs of various types have emerged which cover middle-size molecules and polymers rather than conventional small molecules. Upon those challenges, advanced mass spectrometry (MS)-based technologies will play an increasingly important role, among which the liquid chromatography-tandem mass spectrometry (LC/MS/MS) platform will be powerful as rapid and molecule-based analysis more than ever. The nanoPore Optical Interferometry (nPOI) newly introduced can detect even weak interactions in protein-protein and protein-compound, and can be connected directly to LC/MS/MS for identification of binding molecular species, which will be quite useful for affinity ranking and high-throughput interaction screening. MS-based clinical proteomics utilizing clinical specimens and empowered by advanced bioinformatics can attain both key protein-protein interaction (PPI) networks with major protein players responsible for functional mechanisms of a disease subtype. An integration of those MS-based technologies will deliver a seamless platform of drug development from molecules identified in human clinical specimens. Both somatic mutations and cellular pathways in disease subtypes are mutually intrinsically connected, and so which are needed to be unveiled to understand molecular mechanisms of a disease subtype. In lung cancer, numerous genes acquire mutations which frequently involve EGFR and KRAS, and unavailability of drugs or resistance to the available drugs is the major problem. Therefore, Clinical Proteogenomic approach will become greatly important and will further provide important knowledge to understand the carcinogenetic process and tumor lineages for the benefit of patients with more efficient diagnosis and treatment of these tumors.
High-quality immunoreagents enhance the performance and reproducibility of ubiquitous immunoassays and, in turn, the quality of both biological and clinical measurements. The capacity of an antibody reagent to bind to its target antigen is quantified through the binding dissociation constant (KD) for that pair. One way to generate high quality and high affinity recombinant immunoreagents is by using antibody-phage display. To characterize recombinant antibodies, we introduced a screening affinity electrophoresis format that uses electrophoretic mobility shift assays (EMSAs) designed to operate at high throughput and with minimal sample consumption. The screening workflow is based on microfluidic technologies and integrates with existing laboratory pipelines found in both academic and commercial environments. We design for fast, small volume sample dispensing onto a free-standing polyacrylamide gel (fsPAG) device. In one application, the integrated tool performs 384 concurrent EMSAs of recombinant antibody binding reaction in 30 seconds using a single power source and just two electrodes. We report on characterization of dispersion associated with sample loading, sample injection, and separations. Working with our collaborators at the Recombinant Antibody Network at UCSF, we quantify KD for each of a six-member fragment antigen-binding (Fab) fragment library with 8 replicates of each data point. With collaborators at LabCyte Inc., we explore integration with acoustic liquid handling for droplet dispensing to further enhance precision. After optimization, the microfluidic integrated device requires ~25-fold less sample mass and ~5-fold less time than conventional heterogeneous KD assays; factors that are critically relevant to screening-mode operation. Automated, microfluidic sample handling and measurement tools form a robust and high-throughput basis for biomolecular characterization.

Keywords: Automation, Bioanalytical, Electrophoresis, Lab-on-a-Chip/Microfluidics
Application Code: Bioanalytical
Methodology Code: Microfluidics/Lab-on-a-Chip
Our laboratory has a long-standing interest in microsystems and strategies for capture and analysis of leukocytes. This presentation will highlight some of the recent efforts aimed at employing reconfigurable microfluidic devices, antibody microarrays and sensing microbeads for detecting cytokine release from leukocytes. In addition, this presentation will discuss photodegradable hydrogels for capture and release of specific cells or groups of cells. Such hydrogels may be integrated into microfluidic devices to enable function-based sorting - release of immune cells based on their cytokine production. Tools being described here may enable more nuanced analysis of immune cells and may also offer benefits for small animal research or pediatric immunology where only small blood volume is available.
During an immune response, cells in the lymph node communicate via short-range signaling proteins called cytokines. Current analytical tools can detect only spatially averaged cytokine secretion or temporal snap-shots of activation, missing the highly organized and dynamic nature of the response. We are developing methods to quantify cytokine distribution within a live lymph node slice, while preserving both the structural integrity and intricate dynamics of the tissue. Unlike standard methods that use cell suspensions or fixed tissue, we use live lymph node slices to access temporal and spatial dynamics simultaneously. Testing by flow cytometry confirmed the viability of tissue slices for at least 6 hours post slicing. Results will be presented regarding the design and validation of novel bead-based and cell-based immunoassays for fluorescence-based detection of cytokines inside live tissue. Specific points to be discussed include the design of the affinity reagents, mode of delivery into the tissue, approaches to visualizing tissue topography without perturbing viability, and assessment of spatial heterogeneity in the cytokine response.

**Keywords:** Biological Samples, Fluorescence, Immunoassay, Lab-on-a-Chip/Microfluidics

**Application Code:** Bioanalytical

**Methodology Code:** Microscopy
Microdialysis sampling has been widely used to collect various signaling molecules from primarily the brain. In these contexts, the chemicals used for communicating are quite hydrophilic and easily collected into microdialysis probes. Picking up the chemical communication signature from cells of the immune system is far more complicated for various reasons. First, the cells of the immune system generate numerous chemical classes of chemical communication signals ranging from low molecular weight solutes such as nitric oxide (NO) and superoxide, more hydrophobic signals from the arachidonic acid pathway including the prostaglandins and leukotrienes, and larger proteins including matrix metalloproteinase and cytokine proteins. Our group has had varying interest in these different solutes over many years in different tissues. This talk will describe the unique in vivo chemistry that can be observed with microdialysis sampling and some of the challenges that make this type of in vivo monitoring difficult.

Keywords: Bioanalytical, Biomedical, Sample Preparation, Sampling
Application Code: Biomedical
Methodology Code: Sampling and Sample Preparation
Pharmaceutical processes utilizing flow chemistry and continuous manufacturing are rapidly emerging as the future of commercial manufacturing in the pharmaceutical industry. Small volume continuous manufacturing processes are being implemented in development to demonstrate continuous processes are more efficient, require smaller equipment sets and facilities, and provide higher product quality when compared to batch manufacturing. Successful installations of continuous processes in development with concomitant transfer to manufacturing require online analytical measurement to increase process understanding, ensure process control and product quality. The demand for online analytical information has renewed interest in process analytical technology (PAT), specifically the adaptation of bench top analytical instruments into flexible, portable and rugged online monitoring tools. Our presentation will focus on the implementation of a variety of PAT tools in continuous process development and manufacturing, highlighting specific examples where PAT has provided improved process understanding and real-time decision making to ensure product quality.

Keywords: Pharmaceutical, Process Analytical Chemistry, Process Control, Process Monitoring
Application Code: Pharmaceutical
Methodology Code: Liquid Chromatography/Mass Spectrometry
Building from the recent success in monoclonal antibody based immuno-oncology therapeutics such as Keytruda® have initiated a revolution within the Biopharmaceutical industry. Development efforts to support this initiative include single-use technology, continuous manufacturing, process analytical technology (PAT), IT/MVDA, and real time release (RTR).

During the last twelve years, since the FDA published the PAT guidance for the principles of innovative pharmaceutical development, significant advancements have been achieved for small molecules including real time release. For biotherapeutic development, quality by design provides the framework for building quality into products through detailed molecule, process design and control. Implementation of PAT tools provides a means to improve the process and ensure final product quality through real-time monitoring of critical process and quality attributes (CPPs and CQAs). The majority of previous PAT success has been focused on spectroscopy applications (NIR and RAMAN) which lack specificity and resolution for monitoring protein CQAs. This has initiated a renewed interest in online liquid chromatography (LC) for bioprocess development applications.

Bioprocess case studies developing online LC will be described. This includes upstream and downstream applications for both nutrient and product monitoring in addition to process control. Successful online testing of titer by protein A (Pro A), aggregation by size exclusion (SEC), and charge heterogeneity by ion exchange chromatography (IEX) will be reviewed. Finally, initial efforts utilizing two dimensional liquid chromatography and peptide mapping liquid chromatography mass spectrometry (LC-MS) as multi attribute methods (MAM) for PAT will be introduced.

Keywords: Biotechnology, Liquid Chromatography, Process Control, Protein
Application Code: Pharmaceutical
Methodology Code: Process Analytical Techniques
Recent advances in continuous pharmaceutical and biopharmaceutical manufacturing have renewed interest in acquiring process analytical data in real-time. Analytical instrumentation used to characterize continuous manufacturing must be flexible, portable, and provide information and analytical data rapidly. For biologics, spectroscopic techniques such as IR, Raman, and UV/VIS have been readily utilized to monitor reaction kinetics, nutrients/impurity profiling, and conjugation reaction progress. While these technologies are beneficial for monitoring specific changes or trends in continuous processes, they do not offer the mass sensitivity, specificity, and selectivity that chromatography provides. In this presentation we present our experiences implementing online LC for in-process monitoring of biologics with a common goal: the implementation of rapid process analytics for biopharmaceutical process development and manufacturing. We discuss some of the challenges encountered, specifically with sampling interfaces between process equipment and chromatographic instruments for both sterile sampling and downstream processes. Several applications where online LC has been used to monitor process impurities, upstream nutrients and titer, downstream antibody purification, and means to maintain process control and product quality are also discussed.

**Keywords:** Biopharmaceutical, HPLC, On-line, Process Analytical Chemistry

**Application Code:** Process Analytical Chemistry

**Methodology Code:** Separation Sciences
The production of drug substance via continuous processing is a growing field in the pharmaceutical industry. Continuous flow chemistry offers several advantages to batch processing, including improved scalability, reduction in footprint and energy costs, and the capability to process under extreme conditions. In- and on-line, real-time analytical platforms are essential for successful development and commercialization of continuous flow chemistry, providing the solutions by which processes are monitored and controlled. Integration of analytical techniques is most critical in multi-stage processes as intermediate compounds are not isolated and purified between unit operations. In these cases, it is common to implement multiple analytical techniques that cover a range of response time, selectivity, and sensitivity. A case study will be presented where in-line vibrational spectroscopy and on-line HPLC were implemented at lab and pilot plant scales to support process development and understanding in a multi-stage continuous flow process that contains two chemical transformations and a distillation. An infrared spectroscopic method was developed and implemented in the first chemical transformation to verify consumption of a critical impurity and control state monitoring. The development and implementation of a spectroscopic method for monitoring a final critical quality attribute related to the distillation will also be presented, focusing on the evaluation of Raman and near-infrared spectroscopy. Finally, the development and implementation of an on-line HPLC method for measuring the impurity profile of the final drug substance solution will be discussed.

**Keywords:** HPLC, Pharmaceutical, Process Control, Vibrational Spectroscopy

**Application Code:** Pharmaceutical

**Methodology Code:** Process Analytical Techniques
In January 2010, Archer Daniels Midland was awarded a grant from the Department of Energy (DE-EE0002870) to construct and operate a pilot plant that would demonstrate converting lignocellulosic biomass to ethanol and butyl acrylate. Acrylic acid was generated through oxidation of acrolein, a highly poisonous and flammable chemical. As such, sampling and handling high concentrations of acrolein was undesirable. Through in-line gas chromatography techniques, acrolein formation and conversion was successfully monitored, eliminating exposure risk. The great success in this application lead ADM scientists to explore other areas where process analytical technology could be implemented in the biorefinery, such as monitoring process off-gas for carbon balance closure and rapid analysis of incoming and partially converted feedstocks. The utility of process analytical technology continued to grow throughout the company, with several new application projects being developed jointly between R&D and production plants.

Keywords: Gas Chromatography, Process Analytical Chemistry, Process Control, Spectroscopy
Application Code: Process Analytical Chemistry
Methodology Code: Process Analytical Techniques
Recent Innovations in Nanosensing
Ion Channel Probes (ICPs) for Bio/Chemical Analysis

We describe dual-barrel ion channel probes (ICPs), which consist of an open barrel and a barrel with a membrane patch directly excised from a donor cell. When incorporated with scanning ion conductance microscopy (SICM), the open barrel (SICM barrel) serves to measure the distance-dependent ion current for non-invasive imaging and positioning of the probe in the same fashion of traditional SICM. The second barrel with the membrane patch supports ion channels of interest and was used to investigate ion channel activities. To demonstrate robust probe control with the dual-barrel ICP-SICM probe and verify that the two barrels are independently addressable, current-distance characteristics (approach curves) were obtained with the SICM barrel and simultaneous, current-time (I-T) traces were recorded with the ICP barrel. To study the influence that the distance between ligand-gated ion channels (i.e., large conductance Ca2+ activated K+ channels/BK channels) and the ligand source (i.e., Ca2+ source) has on channel activations, ion channel activities were recorded at two fixed probe-substrate distances (Dps) with the ICP barrel. The two fixed positions were determined from approach curves acquired with the SICM barrel. One position was defined as the “In-control” position, where the probe was in close proximity to the ligand source; the second position was defined as the “Far” position, where the probe was retracted far away from the ligand source. Our results confirm that channel activities increased dramatically with respect to both open channel probability and single channel current when the probe was near the ligand source, as opposed to when the probe was far away from the ligand source.

Keywords: Bioanalytical, Biosensors, Electrochemistry, Sensors
Application Code: Bioanalytical
Methodology Code: Electrochemistry
Recent Innovations in Nanosensing

Nano-Enabled [i]In Vitro[/i] and [i]In Vivo[/i] Diagnostic Tools for Tracking and Treating Disease

Early detection of disease is crucial to improving clinical outcomes for patients. Molecular biomarkers of disease typically include circulating proteins and nucleic acids that are present in low concentrations. This small sample amount results in experimental challenges associated with achieving specific and sensitive biomarker detection through the use of current commercial assays (ELISA and PCR). In addition, current disease biomarkers, such as prostate specific antigen (PSA), have recently been shown to lack the specificity required for unambiguous diagnosis of prostate cancer. The development of new tools that enable rapid, sensitive, and specific detection of trace amounts of biomarkers is required in order to track and manage disease at an early stage.

Certain diagnostic tools, inspired by advances in nanotechnology, are uniquely suited to address the shortcomings of conventional bioassays. Spherical nucleic acids (SNAs), materials composed of gold nanoparticle cores that are functionalized with a dense surface layer of nucleic acids, have enabled highly specific and sensitive detection of disease biomarkers. Bioassays based on SNAs have previously found clinical utility for the detection of infections associated with the bloodstream, respiratory tract, and gastrointestinal tract. This presentation will highlight the synthesis and use of these nanomaterials in high-throughput, microarray-based, miRNA profiling bioassays and also as probes for specific cell sorting and the detection of mRNA transcripts. These nanomaterials represent important clinical tools for studying, tracking, and managing disease.

Keywords: Bioanalytical, Biosensors, Biotechnology, Nucleic Acids
Application Code: Nanotechnology
Methodology Code: New Method
Recent Innovations in Nanosensing

Nanoparticle-Mediated Photothermal Immunosensing Using a Thermometer

Different types of biomolecular quantitation methods have been widely used for various biological applications including disease diagnosis; however, they have limited applications in low-resource settings due to their dependence on expensive and bulky analytical instruments. Recently, the nanoparticle-mediated photothermal effect has attracted increasing attention for photothermal cancer therapy. However, it has not been explored for quantitative biomolecular detection. After systematical studies of several photothermal agents, we for the first time exploited the photothermal effect for quantitative biomolecular detection in multiple immunoassay systems. Those systems utilize either an iron oxide nanoparticle conversion strategy or a new discovery of the photothermal effect from a conventional colorimetric system by our group. Heat generated from the photothermal effect of nanoparticles can be measured by a common thermometer for quantitative biomarker detection. Although thermometers were invented a few centuries ago, they have never been used for quantitative biomolecular detection. The prostate cancer biomarker, prostate-specific antigen (PSA), was used a model analyte and the limit of detection of 1 ng/mL PSA was readily achieved using a thermometer, fully meeting clinical diagnostic requirements. The photothermal effect-enabled thermometer-based immunoassays might open a new field of affordable biomolecular detection to address the challenges faced to conventional biomolecular detection methods in resource-poor settings.

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Keywords: Bioanalytical, Biosensors, Biotechnology, Nanotechnology

Application Code: Bioanalytical

Methodology Code: New Method
Recent Innovations in Nanosensing

Nanosensors for Direct Reading of DNA Damage

Improved therapies that yield more cures and better overall survival for breast cancer patients are needed; women with breast cancer have a 5-year survival rate of 22% (Stage IV) and 72% (Stage III). Doxorubicin, cisplatin, paclitaxel, and tamoxifen are examples of drugs used for treating breast cancer with selection of therapy typically based on the classification and staging of the patient’s cancer. While treatment regimens assigned to some patients may be optimal using the current classification model, others within certain breast cancer sub-types fail therapy. New assays must be developed to determine how a patient’s physiology affects drug efficacy. In this presentation, a novel SMARTChip™ design will be discussed for the isolation and processing of circulating tumor cells (CTCs). The SMARTChip™ quantifies response to therapy using three pieces of information secured from the CTCs; (1) CTC number; (2) CTC viability; and (3) the frequency of DNA damage (abasic (AP) sites) in genomic DNA (gDNA) harvested from the CTCs. The SMARTChip consists of task-specific modules integrated to a fluidic motherboard. Microscale modules are used for CTC selection, CTC enumeration and viability determinations, lysing CTCs, and purifying gDNA. The module to read AP sites is a nanosensor made via imprinting in plastics and contains a nanochannel with dimensions less than the persistence length of double-stranded DNA (~50 nm). Labeling AP sites with fluorescent dyes and stretching the gDNA in the nanochannel allows for direct readout of the AP sites, even from a few CTCs.
In this talk, I will discuss some recent developments in the design and synthesis of silver and gold nanostructures for sensing and imaging applications. In the first half, I will focus on silver nanocrystals with controlled shapes for surface-enhanced Raman scattering (SERS). I will briefly discuss how silver nanocrystals can be synthesized with a variety of shapes, including cubes, spheres, bars, and rods. I will then discuss how these nanocrystals can be assembled into dimers to enable the formation hot spots for SERS detection. Some examples related to SERS sensing and imaging will also be presented, together with a brief discussion on the stability issue and the potential solution. In the second half, I will discuss the synthesis of gold nanocages with tunable absorption in the near-infrared region for photoacoustic tomography (PAT) and related imaging applications.
Five Steps Required for Transition to Enhanced Mechanical Qualification of the Dissolution Apparatus

Whether the dissolution apparatus is calibrated in-house by analysts and metrologists, or if it is outsourced, the spirit of the alternative Enhanced Mechanical Calibration approach for periodic qualification of the apparatus periodic qualification focuses on maintaining the apparatus in top condition and alignment which is seen by industry and regulatory as an improvement over the USP Performance Verification Test. We will discuss five critical steps toward implementation of this process in detail including: certification of components, periodic maintenance, measurements, evaluation of components at time of use and the laboratory procedures required to control significant sources of variability due to vessel quality, deaeration and vibration. Although the eMQ is becoming widely accepted, shortcomings in implementation may generate warning letters for omitting portions of the implementation, not thoroughly documenting the frequency of parameter measurement or failing to properly evaluate the apparatus and components in an ongoing basis. This presentation outlines the requirements in detail.

Keywords: Calibration, Dissolution, Laboratory, Pharmaceutical
Application Code: Pharmaceutical
Methodology Code: New Method
This presentation will summarize the work of the AAPS IVRDTG (In Vitro Release and Dissolution Testing Group) Instrumentation sub team. This sub team is currently working on two projects: 1) the standardization of a biphasic dissolution paddle, and 2) a study to determine the effects of changing vessel inner diameter on dissolution results.

The goal of the first project is to create a universal design for a biphasic paddle to be submitted for consideration by the USP. It is our hope that this new design will help with the standardization of biphasic dissolution testing and improve the comparability of results in academia and industry. Results from paddles of differing dimensions will be compared as a justification for the choice of the current design.

The second project, studying the effect of vessel inner diameter is based on simulated fluid dynamic studies done by Erika Stippler at the USP. In her work, she showed that varying the vessel inner diameter within the allowed USP range (100-106 mm) creates varying hydrodynamic environments. This work will attempt to replicate the earlier simulation work with laboratory results. Testing work using a single bath equipped with vessels of varying inner diameters will be presented and discussed.

Keywords: Dissolution, Instrumentation, Pharmaceutical, Quality Control
Application Code: Pharmaceutical
Methodology Code: Physical Measurements
Cleaning in any GMP laboratory is an important aspect of the analytical experiment. The laboratory must ensure the equipment does not contain residual active pharmaceutical ingredients or impurities that may affect the outcome of any current or future experiments. While this is standard practice for GMP manufacturing operations, common laboratory equipment is often held to less stringent standards. However, the potential man hours lost due investigations for extraneous peaks and contamination can be significant and cause delays in releasing product.

The cleaning of dissolution vessels is somewhat straightforward. We have found in our laboratory that the cleaning of transfer lines is just as important and sometimes overlooked by analysts moving to their next task. Our laboratory has modeled the challenges of cleaning automated dissolution systems using representative soluble and poorly soluble active pharmaceutical ingredients. Poorly soluble drugs often entail the use of surfactants in the dissolution media which also have a potential carryover issue. Using a manufacturing cleaning validation based approach, the validation discussion presented will address the cleaning validation for both sample and media considerations.

Disclosure: All authors are employees of AbbVie. The design, study conduct, and financial support for this research was provided by AbbVie. AbbVie participated in the interpretation of data, review, and approval of the publication.
The goal of the presented studies was to investigate the driving force for drug absorption by introducing an absorption chamber and biomimetic membrane into both compendial and miniaturized dissolution apparati. MacroFLUX and µFLUX devices (Pion) were used to monitor dissolution and absorption processes simultaneously. Both apparati contained absorption chambers either inserted into USP II dissolution bath (MacroFLUX) or attached side-by-side in the miniaturized bath (µFLUX) and separated from the dissolution chamber by artificial lipophilic membrane (Double-Sink PAMPA model, Pion). Concentration monitoring in both dissolution and absorption chambers was enabled through fiber optic UV probes connected to the Rainbow instrument (Pion). The ratio of the flux from FeSSIF media to the one from FaSSIF was used as an indicator of positive, negative or neutral food effect. It was predicted using µFLUX apparatus that food effect of model drug Danazol should be positive with approximate absorption fraction increase of 4 folds. A similar (3 fold) increase was reported in humans.

MacroFLUX apparatus showed differences not only in dissolution, but also in flux behavior between brand and generic formulations for model drugs Naproxen and Phenazopyridine. The flux studies indicated potential drug-drug interaction risk for Phenazopyridine when co-administered with pH-modifying agents where the pH of the stomach reaches pH 6.5. Decreased flux for a generic formulation of model drug Telmisartan compared to its brand formulation explained differences in bioequivalence studies for the generic formulation. The dissolution experiments alone could not explain the shift in C[sub]max[/sub] and T[sub]max[/sub] for the generic formulation while changes in the flux provided quantitative estimation for these changes. The case studies demonstrate the potential of the flux device to reduce the burden of in vivo measurements by providing prediction using relatively simple in vitro method.

Keywords: Dissolution, Drugs, Pharmaceutical, UV-VIS Absorbance/Luminescence
Application Code: Pharmaceutical
Methodology Code: UV/VIS
Modern pharmaceutical formulation development has become more and more challenging. The majority of new drug candidates are poorly soluble, either a BCS class II or class IV. Sophisticated formulation strategies must be implemented to enhance drug solubility, absorption and maximize drug bioavailability while balancing product stability to ensure appropriate shelf-life. Challenges also come from demanding timelines, limited quantities of drug substance, and limited in-vivo data in early stages of drug development. Therefore, effective and fast selection of advanced drug delivery approaches and appropriate formulation prototypes becomes critical, demanding the use of advanced in-vitro dissolution technologies for rapid screening and evaluation of the drug formulation performance for better understanding of biopharmaceutics risks.

To facilitate drug formulation development, the in-vitro dissolution testing methods used for the prototype evaluation must be biorelevant. They are not the same as those used for quality control. Their purpose is to speed up the evaluation by saving or using less pre-clinical animal models, while obtaining a biorelevant rank order of drug release, and screening out poor performers. Biorelevant dissolution can be performed by using biorelevant media to simulate gastrointestinal fluids, or by using biorelevant apparatus to simulate gastrointestinal hydrodynamics. In this talk, several real cases of formulation screening and evaluation using advanced biorelevant dissolution technologies and devices will be presented, including application of biphasic dissolution, transfer model and micro dissolution. The pros and cons of these technologies will also be discussed.

**Keywords:** Dissolution, Fiber Optics, Method Development, Pharmaceutical

**Application Code:** Pharmaceutical

**Methodology Code:** New Method
Traditionally, analyzing more than one active pharmaceutical ingredient (API) with UV spectrophotometry poses a challenge as both species often absorb over the same spectral region, causing deviations from Beer’s Law. This linear relation between absorbance and the absorbing species is the basis for calculating concentration values based on the measured absorbance at a specific wavelength. Separation techniques such as HPLC are often reverted to when analyzing mixture samples with more than one API due to the concentration calculation errors caused by the spectral overlap. However, Multicomponent Analysis (MCA) software and complete spectral and temporal profiles collected using a fiber optic UV dissolution analyzer overcome these obstacles. This is accomplished using the Classical Least Squares form of Multiple Linear Regression to analyze the two spectrally overlapping components. The algorithm uses a calibration matrix of extinction coefficients to calculate component concentrations in an unknown mixture. These are derived from a training set comprised of the spectra of multiple standard solutions. The MCA module can also be used to measure one API in the presence of strong interference from excipients, coatings, etc. This study demonstrates the MCA algorithm’s capability, used in tandem with in-situ fiber optics, to accurately monitor and quantify the dissolution profile of a commercial product containing two APIs, eliminating the need to draw samples for HPLC analysis.

Keywords: Chemometrics, Dissolution, Quantitative, UV-VIS Absorbance/Luminescence
Application Code: Pharmaceutical
Methodology Code: UV/VIS
Focused on the experiences of Robert E. Finnigan, co-founder of Finnigan Instruments, the commercialization of quadrupole mass spectrometry in the 1960s will be reviewed, with particular attention to the contexts of microelectronics, computing, and process control. The commercialization of GC/MS/DS [Data System] in the late 1960s will be surveyed, and the centrality of the life sciences and medicine as a context will be explored. Historian David C. Brock will deliver the paper, which will feature illustrative video commentary from Robert E. Finnigan.

**Keywords:** Biomedical, Computers, Mass Spectrometry, Quadrupole MS

**Application Code:** Biomedical

**Methodology Code:** Mass Spectrometry
<table>
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<th>Session Title</th>
<th>From Discovery to Precision Medicine: Mass Spectrometry Through the Years and Beyond</th>
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<tr>
<td>Abstract Title</td>
<td>A Brief History (1974-2016) of Mass Spectrometry Instrumentation Driving Cutting-Edge Biological Research that then Stimulates Development of New Mass Spectrometry Instrumentation</td>
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| Primary Author | Donald F. Hunt | University of Virginia |

**Co-Author(s)**

**Abstract Text**

This lecture will describe a number of innovations that resulted from a 42 year (and counting) collaboration with the Finnigan Corporation (now ThermoFisher Scientific). Topics include: (1) PPINICI and the conversion dynode EM for quantitation of drugs at the attomole level (1976-78); (2) construction of the triple quadrupole mass spectrometer for peptide sequence analysis of permethylated peptides (1978-80); (3) construction of a Quadrupole FTMS instrument for high resolution analysis of peptides and sequence analysis by photodissociation (1984-85); (4) modification of TSQ instruments for ESI and both CE and microcapillary HPLC (1990-91); (5) sequence analysis of the first class I and class II MHC peptides by mass spectrometry and modification of the QFTMS instrument to facilitate identification of antigenic peptides recognized by T-cells that were then sequenced on a TSQ instrument (1992-99); (6) construction of the first LTQ-FTMS instrument for peptide sequence analysis (2002); (7) modification of an LTQ instrument to perform ETD and IIPT (2004); (8) Development of front end ETD ion sources for the Velos-Pro Orbitrap instruments that make it possible to sequence posttranslationally modified, intact proteins on a chromatographic time scale by a combination of ETD, parallel ion parking, and IIPT (2010-16).

**Keywords:** Bioanalytical, Biological Samples, HPLC, Proteomics  
**Application Code:** Bioanalytical  
**Methodology Code:** Mass Spectrometry
This talk presents first-hand a short but eventful history of Orbitrap mass spectrometry, from laying down the first principles to its current status as the leading mass spectrometric technique for high-resolution, high mass accuracy quantitative analysis. Originating from an ideal (and therefore never realized) Kingdon trap, this analyzer can provide high performance analytical characteristics only when it is highly integrated with the ion injection process. The advent of pulsed injection from an external ion storage device allowed the Orbitrap analyzer to enter mainstream mass spectrometry, initially as a part of a hybrid instrument.

Since its introduction the utility of the analyzer has been extended by coupling with additional capabilities such as quantitative analysis, new fragmentation methods, different vacuum and ambient ion sources, imaging and ion mobility. These capabilities are exemplified for three major families of Orbitrap-based instruments, with numerous new modes of operation enabled by parallelization of detection and ion processing, and intricate coordination with different ion-optical devices, especially with a quadrupole mass filter. New modes of data-independent, targeted and top-down acquisitions are overviewed.

In conclusion, future trends and perspectives of Orbitrap mass spectrometry are discussed, including its expansion into emerging areas of mass spectrometric analysis. It is shown that Orbitrap-based mass spectrometers possess compelling potential as an (ultra-) high resolution platform not only for high-end proteomic applications but also for screening, trace and targeted analysis by LC/ and GC/MS.

Keywords: Gas Chromatography/Mass Spectrometry, Liquid Chromatography/Mass Spectroscopy, Mass Spectro Application Code: Genomics, Proteomics and Other 'Omics Methodology Code: Mass Spectrometry
Biomonitoring (i.e., trace level measurement of environmental chemicals or their metabolites in biospecimens) provides a quantitative measure of the amount of a given chemical present in the human body after integrating all sources and routes of exposure. Biomonitoring methods, rooted in their analytical chemistry foundation, must be sensitive, selective and specific, and accurate and precise at trace levels. In the last few decades, thanks in part to advances in robotics and analytical chemistry techniques, such methods, particularly isotope-dilution mass spectrometry, are now common in many laboratories and the use of biomonitoring has increased considerably. Biomonitoring programs are particularly useful for investigating human exposure to environmental chemicals. One of these programs, the National Health and Nutrition Examination Survey (NHANES) is conducted annually by the Centers for Disease Control and Prevention. NHANES collects data on the health and nutritional status of the general U.S. population, as well as biological specimens which can be used to assess exposure to select chemicals. NHANES biomonitoring data have shown that exposure to some environmental chemicals is prevalent. NHANES data also suggest variability in exposure by sex, age, and race/ethnicity, all of which probably reflect lifestyle differences. This presentation will provide an overview of recent developments in the design and interpretation of biomonitoring strategies, as well as the public health uses of NHANES biomonitoring data (e.g., establish reference ranges, provide exposure information for risk assessment, monitor exposure trends).
A greater understanding of the effect of surface defects on the electrochemical reactivity of graphitic materials is important both fundamentally and practically. In this work, we assess the kinetic effects of surface defects by comparing the intrinsic electrochemical reactivity of highly defected graphite with that of highly ordered pyrolytic graphite. Previous studies demonstrated that electron-transfer kinetics at glassy carbon was immeasurably high by cyclic voltammetry even at 500 V/s, where an Ohmic potential drop became significant in the bulk carbon material. In this study, we employ nanogap voltammetry based on scanning electrochemical microscopy (SECM) to measure the extremely high reactivity of defected graphite surfaces under higher mass transport conditions. Specifically, electron-beam deposited carbon is investigated as a new model of highly defected graphite. In comparison with glassy carbon, the flatter surface of electron-beam deposited carbon (a mean-root-square roughness of ~1 Å) is not only advantageous for the formation of an extremely narrow gap under an SECM tip, but also more defected as demonstrated by its covalent modification with a closely packed molecular layer for molecular electronics applications.

Keywords: Electrochemistry, Nanotechnology
Application Code: Nanotechnology
Methodology Code: Electrochemistry
**Session Title**: SEAC: The Student Session in Electroanalysis  
**Abstract Title**: Convolution-Based Removal of Non-Faradaic Background Current in Fast-Scan Cyclic Voltammetry Recordings

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<th>Primary Author</th>
<th>R Mark Wightman</th>
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<td>University of North Carolina at Chapel Hill</td>
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**Abstract Text**

Fast-scan cyclic voltammetry (FSCV) at carbon-fiber microelectrodes has proven a valuable tool for monitoring sub-second in vivo fluctuations in electroactive neurotransmitters. In its practice, digital background subtraction is routinely used for signal resolution due to the large background current generated at the high voltammetric scan rates employed. Such an approach, however, suffers from signal interferences originating from background instability, often due to changes in the impedance characteristics of the electrochemical cell. Consequently, the type of data obtainable from the technique is limited to differential concentration changes over short time periods (i.e. seconds to minutes) in stable ionic environments.

To circumvent this issue, a new measurement protocol has been developed for prediction and removal of non-Faradaic component of the FSCV background signal. The approach uses a small-amplitude step, placed before the triangular FSCV sweep, to probe the capacitive state of the electrode. This information is then used with digital convolution for estimation and subtraction of the non-Faradaic component of the voltammetric response. In this talk, the fundamentals of this technique will be described, along with the experimental parameters that facilitate its use (e.g. instrumentation and waveform parameters). Additionally, successful in vivo application of this will be presented, including monitoring of catecholamines during severe ionic fluctuations (i.e. during a spreading depression event) and over previously inaccessible time windows.

**Keywords**: Bioanalytical, Electrochemistry, Neurochemistry, Voltammetry

**Application Code**: Neurochemistry

**Methodology Code**: Electrochemistry
Scanning electrochemical microscope (SECM) is used to perform 3D chemical mapping of surfaces ranging from biomaterials to biological systems. The SECM gives information about the local chemical concentration with help of ultra micro electrode (UME) probes. A new type of potentiometric sensor, which is a carbon paste based ion selective microelectrode (ISME) has both potentiometric and amperometric functions. The amperometric function was primarily used to perform amperometric probe approach curve with the SECM. The ISMEs for Ca$^{2+}$, K$^+$ and pH gave Nernstian slopes and a broad dynamic range. The ISMEs had a tip diameter of 25 µm and a fast response time of 0.2s. The ISMEs for Ca$^{2+}$ and pH were used to obtain 3-dimensional chemical imaging of biomaterials as well as biological process. Local pH and Ca$^{2+}$ concentration of pure Bioactive Glass-dental composite was characterized. The data showed that there was an increase in Ca$^{2+}$ concentration accompanied by neutralization of the acidic pH, to which the BAG was exposed to. The pH and Ca$^{2+}$ sensors where also used to find the local chemical concentration of BAG/Resin dental composites containing different sizes of BAG particles. The data was used to find the optimum composite with good mechanical strength, ideal chemical concentration and good distribution to prevent bacterial growth. In another study the rate of depletion of Ca$^{2+}$ on top of a bacteria performing urea hydrolysis was measured. The characterization of the dual function sensor with impedance, SWV, CV and other techniques will also be presented.

**Keywords:** Bioanalytical, Biosensors, Electrochemistry, Material Science

**Application Code:** Bioanalytical

**Methodology Code:** Electrochemistry
Micro albumin and kidney failure is a worldwide issue that calls for critical attention of effective biosensors for early diagnosis. The advent of virus-biosensor technology presents a promising and non-invasive solution by quantitatively detecting human serum albumin (HSA) using electrical resistance-based measurements. Virus-composite films are prepared by incorporating M13 bacteriophage (phage) into a conductive polymer, poly 3,4-ethylenedioxythiophene (PEDOT), on the surface of millimeter-scale gold electrodes using electrodeposition. M13 bacteriophage are modified to display peptides for specific, high affinity binding to HSA. The electrochemical impedance of virus-PEDOT films increases upon exposure to HSA within a 5-minute incubation period. Changes in the real component of the impedance show virus-PEDOT films follow a dose-response relationship to HSA over a medically relevant concentration range of 100 nM to 5 µM with a coefficient of variance ranging from 2–8%.
As electrical energy storage devices (EES), lithium-ion batteries (LIBs) are considered to have the highest energy and power densities. They are currently used in numerous applications including hybrid and electric vehicles, consumer electronics, grid leveling and other industrial applications. Conversion materials, as opposed to conventional intercalation/insertion compounds, show great promise because of their potentially high theoretical capacities. The goal of studying this class of materials is to develop a detailed mechanistic understanding of the reactions involved. Such understanding can then lead to the development of new materials as well as the improvement of existing ones. Nickel molybdate (NiMoO4) is a high capacity anode material whose conversion reaction is believed to yield reduced metals and lithium oxide. This study is designed to establish if this is, in fact, the case.

NiMoO4 was prepared by chemical precipitation of the ammonium nickel molybdate salt and subsequent annealing in air to from the desired product. The material was cycled and tested electrochemically to confirm the electrochemical performance. Operando X-ray diffraction (XRD) and X-ray absorption spectroscopy (XAS) measurements were performed at the Cornell High Energy Synchrotron Source (CHESS). From the starting monoclinic structure, there is visible structure degradation and amorphization upon cycling. There is also a significant increase in the electrolyte background, indicating that there is likely electrolyte decomposition. Operando XAS measurements indicated that the final oxidation state of molybdenum was in a partially reduced state while the nickel appears to be fully reduced. These observations are consistent with the above-mentioned mechanism.

**Keywords:** Electrochemistry, Energy, Spectroscopy, X-ray Diffraction

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

**Methodology Code:** X-ray Techniques
**Session Title:** SEAC: The Student Session in Electroanalysis  
**Abstract Title:** Electrolysis of Ammonia Using Earth-Abundant Materials  
**Primary Author:** Daniel J. Little  
**Affiliation:** Michigan State University  

**Abstract Text:**  
Much of the global effort toward solar energy conversion to fuels has involved splitting water to obtain molecular hydrogen. Unfortunately, hydrogen gas has a relatively low energy-per-volume, and extreme cryogenic temperature and pressure is needed to bring hydrogen to a condensed state. An interesting choice for an alternative solar fuel that circumvents these setbacks is ammonia. Synthesis of ammonia (largely via the Haber-Bosch process) is already among the largest chemical industrial processes in the world, and as a result, the infrastructure for ammonia storage and transport already exists. Ammonia can be easily liquefied under 10 bar of pressure, which leads to a 3-fold higher energy density than hydrogen gas in state-of-the-art carbon fiber cylinders.[1,2] While ammonia can theoretically be electrolyzed to nitrogen and hydrogen using less than 0.1V, (less than 10 percent of the potential needed for water electrolysis), catalysts are needed for both the anodic and cathodic half reactions to eliminate the need for large (greater than 1.5V) overpotentials.[3] This presentation will highlight the pitfalls of ammonia oxidation on conventional electrode materials, and explore how new, Earth-abundant materials can be employed to improve anodic efficiency. A discussion of the unexpected mechanistic pathway for di-hydrogen generation in liquid ammonia will be included,[3] as well as our efforts to minimize the resulting cathodic overpotential using new heterogeneous catalysts for this reaction.

**References**  

**Keywords:** Electrochemistry, Electrodes, Energy, Fuels\Energy\Petrochemical  
**Application Code:** Fuels, Energy and Petrochemical  
**Methodology Code:** Electrochemistry
Redox active polymers (RAPs) are a new class of energy material for use in size-exclusion non-aqueous redox flow batteries that were pioneered by our groups.\cite{1} Despite RAPs displaying excellent charge storage properties, the electrochemical pathways are only recently becoming understood. Analysis of viologen and ferrocene RAPs revealed that RAP reactivity obeys a CE mechanism, whereby preceding chemical steps (C) are associated with electron transfer (E).\cite{2} Although this study was comprehensive, the critical role of ionics on the observed electrochemistry was underplayed and was first revealed when studying a nitrostyrene based RAP.\cite{3} Here, the crucial selection of supporting electrolyte ions modulated the observed faradaic current and electrochemical kinetics by orders of magnitude.

Electron hopping through the RAP coils was identified as a potential rate limiting step. A second generation of RAPs were designed which maximized self-exchange reactions by modifying the inter-pendant distance. However, enhanced self-exchange without improving ionic accessibility was not sufficient to improve RAP reactivity. Beyond the identity of supporting electrolyte ions, the concentration thereof has revealed unique electrochemical dependencies in RAPs that are not observed in their monomer counterparts. Careful consideration of the supporting electrolyte concentration and Debye lengths are needed to observe optimal reactivity of RAPs. Advanced in-situ analysis via EPR and TEM methodologies promise to provide clarity on the charge propagation mechanisms of RAP particles.

References:
\cite{1} Nagarjuna, et. al., \textit{J. Am. Chem. Soc.} \textit{2014}, 136 (46), 16309-16316.
\cite{3} Burgess, et. al., \textit{Analyst} \textit{2016}, 141, 3842-3850.

Keywords: Electrochemistry, Energy, Polymers & Plastics, Spectroscopy
Application Code: Polymers and Plastics
Methodology Code: Electrochemistry
Chronic wounds have a significant negative impact on the quality of life of affected individuals. Treatment of these wounds would benefit from in situ monitoring of the wound environment. To this end, a “smart bandage” is a desirable technology to report the status of a wound healing. A “smart bandage” would consist of electrochemical sensors capable of selectively detecting the oxygen level, pH, temperature, moisture in the dressing and the biomarkers of the bacterial infection, such as pyocyanin and uric acid. A small-size, cost-effective and reproducibly-prepared electrode material is needed for such a bandage. We are investigating carbon nanotubes in a combination with the ink-jet printing technology as a possible electrode/sensor material. We are currently focused on the first level of the smart bandage engineering, and that is the basic electrochemical properties of the electrodes, their ability to detect biomarkers of infection and their stability in a model wound environment.

In this presentation, the electrochemical properties of the ink-jet printed carbon nanotubes (CNT) electrodes, as studied by cyclic voltammetry, will be discussed. The basic electrochemical properties will be reported on as will be the electrode performance for one biomarker of infection, pyocyanin. The physical stability of the CNT electrodes in a phosphate buffer (pH 7.2) and a wound fluid mimic (pH 7.2) at 37 °C will also be discussed. Finally, the detection figures of merit for pyocyanin in the wound fluid mimic will be reported.

Keywords: Electrochemistry, Sensors, Voltammetry
Application Code: Bioanalytical
Methodology Code: Electrochemistry
Zebrafish have become a popular tool for the study of various neurological disorders. Previously, our group and others have demonstrated the suitability of zebrafish as a useful model system for the measurement of neurotransmitter release with fast-scan cyclic voltammetry. Here, we demonstrate the measurement of dopamine release and uptake in whole zebrafish brain. The whole brain preparation is useful as a model system because tissue damage associated with obtaining brain slices is minimized while the neuronal pathways remain intact. Moreover, due to its small size, zebrafish whole brain can remain viable in a perfusion chamber for longer periods of time compared to brain slices. The optimization of stimulus parameters, important differences between the pharmacological treatments, and the remote stimulation of pathways will be discussed.

Keywords: Bioanalytical, Biosensors, Electrochemistry, Microelectrode
Application Code: Bioanalytical
Methodology Code: Electrochemistry
Electrochemical aptamer-based (E-AB) sensors are widely used in a myriad of point-of-care applications. Their rapid response, accuracy, and selectivity towards its specific target make them the new promising biorecognition elements. To date, most of the E-AB sensors uses deoxyribonucleic acid (DNA) aptamers as biological recognition element. Despite this dominance, ribonucleic acid (RNA) aptamers exhibit exquisite characteristics resulting from the presence of a 2’-OH group and non-Watson-Crick base pairing. These peculiarities allow any RNA aptamer to fold in more complex 3-D structures than their DNA counterparts, and consequently, exhibit better binding ability to small molecules. Unfortunately, RNA aptamers elements are more prone to degradation by nucleases. For this reason, RNA-based sensors are scarce. The aim of this work is to create a robust method to protect RNA E-AB sensors from nuclease-rich environments. A collagen hydrogel scaffold was used to protect the photolithographically-patterned E-AB sensor fetal bovine serum (FBS). Experimental conditions showed a 2 mg/mL hydrogel with 2 \( \text{mg}^2 \) pore size control the signal reduction by enzymatic degradation processes. By combining the aforementioned concentration and an approximate thickness of 4 mm, results showed a 24% signal reduction attributed to the physical interaction between the hydrogel matrix and the aminoglycoside antibiotics-RNA E-AB sensor. This sensor was used to the quantification of the aminoglycoside antibiotic tobramycin (Tob) in 100% undiluted FBS serum and incubated in this matrix for approximately 60 min. RNA sensors showed no electrochemical signal degradation after this incubation period. The sensor response was fit into a Langmuir-type binding isotherm by plotting the average percentage of signal change vs. Tob concentration. The obtained binding affinity value for this RNA sensor embedded to a 4mm thick 2 mg/mL collagen was \( K_d = 0.12344 \). This effort demonstrated its usefulness protecting RNA E-AB sensors.

Keywords: Bioanalytical, Biosensors, Electrochemistry, Nucleic Acids

Application Code: Bioanalytical

Methodology Code: Electrochemistry
Microneedle Arrays are defined as devices with needle-like projections with lengths typically up to 1mm and diameter in the order of microns. They are minimally invasive and simplistic devices, foreseeing a progressive divergence from the field of drug delivery and vaccination into the realm of diagnostic and/or point-of-care (POC) devices. Such systems are highly relevant for, (1) diseases that require frequent monitoring such as diabetes, (2) rapid diagnosis of infectious diseases e.g. Dengue Fever and (3) for differential diagnosis of complex diseases. Recent developments in microneedle (MN) based POC systems have seen the emergence of in vivo biosensing MN’s that would enable real time and continuous detection of physiologically relevant molecules such as dopamine, uric acid and nitric oxide. However, progress in this area is limited by multi-step processing and lengthy fabrication times. Thus, we showcase micromoulding as a facile fabrication strategy for developing Carbon-Nanotube/Poly (lactic) acid composite MNs; emphasizing the fabrication steps involved and their influence on MN electrochemical behavior. Mechanical assessment of the MNs have shown the axial load and sheer failure forces to be strong enough to withstand resistive forces during skin insertion. Electrochemical characterization of these MNs have demonstrated their ability to sample physiologically relevant molecules such as ascorbic acid at low concentrations. We further report on the ability of these MNs to sample from complex tissue environments.

Keywords: Biosensors, Electrochemistry, Nanotechnology, Voltammetry

Application Code: Bioanalytical

Methodology Code: Electrochemistry
Studies of neurotransmitters are critical for a better understanding of how our brain works. Fast-scan cyclic voltammetry (FSCV) is the most popular electrochemical technique for the in vivo detection of electroactive neurotransmitters and neurochemicals with high temporal resolution. To further improve the detection selectivity, sensitivity, and spatial resolution, carbon nanomaterial based microelectrodes are applied to enhance FSCV for the neurotransmitters detection. Synthesis/fabrication of several novel carbon nanomaterials, the effect of different surface modifications, as well as systematic comparison of different carbon nanomaterials and different surface modifications will be introduced and discussed, to answer both fundamental and practical considerations for implementations focusing on neurotransmitters.

For the practical application of electrodes, we successfully applied and investigated several carbon materials (CNT- metal, CNT yarn, and CNT fibers made by different polymers), surface treatments (laser etching, O2 plasma treatment, and anti-static gun treatment), and advanced sensor fabrication methods (3D printed mold and 3D printed electrode substrate), for in vivo neurotransmitter monitoring. For the fundamental studies, we developed a better understanding of how surface properties lead to electrochemical properties. A systematic comparison of different carbon nanomaterials and different surface modifications provides a useful structure to evaluate which new nanomaterials would be good as electrochemical sensor and which electrode a neuroscientist might choose for different experiments. Overall, a better understanding and application of carbon nanomaterials sensors for neurotransmitters detection will be introduced, which could facilitate the neuroscience study of behaviors and neurodegenerative disease and promise to be a fruitful research area for years to come.

Keywords: Electrochemistry, Electrode Surfaces, Materials Characterization, Neurochemistry

Application Code: Bioanalytical

Methodology Code: Electrochemistry
Bioanalytical - Electrochemistry

Measurement of Pyocyanin from [i]Pseudomonas Aeruginosa[/i] in Polymicrobial Environments Using Electrochemical Sensors

A major cause of death among hospitalized patients is infection acquired in the hospital setting, with Pseudomonas aeruginosa being one of the most prevalent hospital-acquired bacterial pathogens. This bacterium produces a unique, redox-active molecule known as pyocyanin. Because pyocyanin can be detected using electrochemistry, it serves as a useful biomarker for identification and detection of this opportunistic pathogen. While previous research has electrochemically detected P. aeruginosa’s production of pyocyanin in complex media environments, this study addresses the need to understand how P. aeruginosa behaves when co-cultured with other bacterial pathogens by electrochemically monitoring P. aeruginosa’s production of pyocyanin in polymicrobial samples. Polymicrobial infections are prevalent among hospitalized patients.

Liquid cultures of the most common clinically-relevant bacteria (Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, Staphylococcus epidermidis, and Enterococcus faecalis) were grown overnight at 37 °C in lysogeny broth or trypticase soy broth growth media. From these stock cultures, different co-culture combinations were inoculated into fresh media. Production of pyocyanin was monitored every hour for the first 24 hours and then every 6 hours afterwards for a total of 3 days. Measurements of current versus applied voltage were recorded for each of the different bacterial samples. Using a calibration curve of pyocyanin standards, the measured current can be correlated to the pyocyanin concentration in the sample. The results from this study demonstrate that P. aeruginosa produces pyocyanin at similar rates, regardless if other bacterial pathogens are present.

Abstract Text

Keywords: Bioanalytical, Detection, Electrochemistry, Voltammetry
Application Code: Bioanalytical
Methodology Code: Electrochemistry
Human liver microsomes (HLMs) are the major membrane-bound drug metabolizing enzymes consisting of cytochrome P450 (P450) enzymes and their redox partner protein, CPR. They act as an ideal in-vitro system to study pharmacokinetics, drug metabolism, and drug-drug interactions for new drug development. Therefore, developing microsomes-based biosensors and bioreactors have immense significance in green synthesis of drug metabolites and stereoselective chemicals, and biosensing. Our prior studies showed that HLMs adsorbed on high purity graphite electrode and edge plane pyrolytic graphite electrode showed better film stability and catalytic currents than smooth glassy carbon and gold electrodes. Moreover, this prior work allowed design of one-step rapid HLM platform for voltage-driven, NADPH-free drug metabolism and inhibition assays. Recently, we also demonstrated the advantage of using carbon nanotubes with HLMs for enhanced drug metabolite production and reusability. Bactosomes are membranes containing a specific human P450 isoform coexpressed with CPR. Our current objective is to examine electrochemical properties of biologically active films of bactosomal P450 3A4 expressed with varying proportions of CPR. Direct electrochemical and electrocatalytic properties of new bioelectrodes featuring bactosomal P450 3A4 enzyme films will be discussed.

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

References:

Keywords: Bioanalytical, Electrochemistry
Application Code: Bioanalytical
Methodology Code: Electrochemistry
Bacteria detection is crucial in prevention, identification and treatment of diseases and pathogenic outbreaks[1]. Level of bacteria in most samples is low so that conventional methods rely on incubation, amplification steps, sophisticated detection devices or a combination of those[2].

Here, we report an alternative approach based on detection of electrochemically induced light emission called electrochemiluminescence (ECL). The reverse assay we proposed, exploits the fact that bacteria can disturb the ECL. The method does not require electrode modification or labeling. The ECL system consists in ruthenium tris(2,2'-bipyridine)(II) as luminophore and tripropylamine as co-reactant. For development purposes, we used the non-pathogenic [i]E. coli[/i] K12. The emission can be detected by naked eye or using ubiquitous devices like mobile phones. In the absence of bacteria, ECL is generated (Fig. 1a). Upon addition of bacteria, however, the intensity decreases or disappears completely depending on the amount of bacteria present (Fig. 1b). The presence of bacteria also caused a decrease of the peak current of the corresponding cyclic voltammograms.

Our results exhibited excellent repeatability and our ongoing research focuses on maximizing the sensitivity targeting to answer the challenge of detecting a single bacterium without lengthy pre-concentration culture and then translate the platform to the detection of specific pathogenic bacteria.

References:

Keywords: Bioanalytical, Electrochemistry, Food Safety, Luminescence
Application Code: Bioanalytical
Methodology Code: Electrochemistry
A Cooperative-Binding Split Aptamer Assay for Rapid, Specific and Ultra-Sensitive Fluorescence Detection of Cocaine in Saliva

Sensors employing split aptamers that reassemble in the presence of a target can achieve excellent specificity, but the accompanying reduction of target affinity mitigates any overall gains in sensitivity. We for the first time have developed a split aptamer that achieves enhanced target-binding affinity through cooperative binding. We have generated a split cocaine-binding aptamer that incorporates two binding domains, such that target binding at one domain greatly increases the affinity of the second domain. We experimentally demonstrate that the resulting cooperative-binding split aptamer (CBSA) exhibits higher target binding affinity and is far more responsive in terms of target-induced aptamer assembly compared to the single-domain parent split aptamer (PSA) from which it was derived. We further confirm that the target-binding affinity of our CBSA can be affected by the cooperativity of its binding domains and the intrinsic affinity of its PSA. To the best of our knowledge, CBSA-5335 has the highest cocaine affinity of any split aptamer described to date. CBSA-based assay also demonstrates excellent performance on target detection in complex samples. Using this CBSA, we achieved specific, ultra-sensitive, one-step fluorescent detection of cocaine within fifteen minutes at concentrations as low as 50 nM in 10% saliva without signal amplification. This limit of detection meets the standards recommended by the European Union’s Driving under the Influence of Drugs, Alcohol and Medicines program. Our assay also demonstrates excellent reproducibility of results, confirming that this CBSA-platform represents a robust and sensitive means for cocaine detection in actual clinical samples.

Keywords: Bioanalytical, Drugs, Nucleic Acids, Sensors
Application Code: Bioanalytical
Methodology Code: Fluorescence/Luminescence
# Reversible Distribution of G Protein [beta][gamma]9 Based Assay for Real-Time Quantification of GPCR and G Protein Function in Living Cells

G protein coupled receptors (GPCRs); being the largest family of cell surface receptors in eukaryotic genomes, control a variety of cellular and physiological processes and have been involved in a majority of pathological conditions. They sense and respond to a wide range of ligands ranging from small molecules to proteins. Current assays to measure ligand induced activation of GPCRs-G proteins are time consuming, indirect and expensive. Therefore, an efficient method to measure GPCR-G protein activation is required to understand the molecular basis of GPCR and G protein activity and to identify new ligands for GPCRs. We have developed a live cell imaging based assay to quantify the extent of GPCR-G protein activation in real time. Using confocal microscopy, we measure the GPCR activation induced G protein heterotrimer dissociation and resultant G protein [beta][gamma] subunit translocation from the plasma membrane to internal membranes and quantify the change in G[beta][gamma] distribution after exposing cells to different ligand concentrations. This assay also works with low magnification (10X) epi-fluorescence microscopy and allows qualitative measurement of GPCR activation in live cells, making it high-throughput-adaptable. We also use this assay to measure subcellular GPCR-G protein activation after triggering such activities using optogenetic control of signaling. In summary, our reversible G[beta][gamma]9 assay allows subcellular to cellular and qualitative to quantitative measurement of GPCR-G protein function in living cells in real time. Therefore, our assay can be used for GPCR deorphanization as well as for screening ligands for GPCRs.

**Keywords:** Bioanalytical, Biosensors, Fluorescence, Imaging

**Application Code:** Bioanalytical

**Methodology Code:** Fluorescence/Luminescence
Our long term goal is to develop a set of optical nanosensor “tattoos” paired with a modified cell phone so patients could monitor their own health status. In particular, our chloride nanosensor could track extracellular chloride levels for cystic fibrosis patients who suffer from impaired chloride transport. This would empower patients to continuously monitor their own chloride levels and to assess their individualized response to therapy. In this work, we have developed fluorescent ratiometric nanosensors for measuring chloride ions. The key components of our nanosensor platform include chromoionophore, chloride ionophore, additives and fluorophore. Based on the ion co-extraction mechanism, our nanosensors are fabricated in two forms: nanoparticles with high brightness and fast response, and nanofibers with potential increased stability in vivo. Both nanoparticle and nanofiber sensors show high sensitivity to chloride ions in the physiological range (90mM to 110mM), exhibiting 38% and 44% changes per log concentration respectively. Both sensor forms display high selectivity to chloride in the presence of key interfering anions in the blood such as iodine, bromide, carbonate and phosphate. We also demonstrate the sensors implanted in a murine cystic fibrosis model with a real-time response to systemic change in chloride level. Future work will focus on nanosensor optimization and characterization for quantitative measurement in vivo.
Analyzing [*Drosophila melanogaster*] Hemolymph with Different Sampling Techniques, Capillary Electrophoresis, and Fluorescence Cell Sorting

The hemolymph of the animal [*D. melanogaster*] is a chemically complex, biological fluid that plays blood-like and extracellular fluid roles physiologically. Previously, amino acid and small peptide content has been characterized from both individual larval and adult flies. Questions remain regarding any impact on hemolymph chemical content due to processes used for sampling including the physiological state of the fly, the anatomical location of hemolymph collection, and the derivitization procedure.

To understand differences in the hemolymph composition, three different sampling techniques were explored. These techniques include a device which allows no anesthesia to be used, a method of anesthetization via carbon dioxide, and a cold shock anesthesia method. Independent of the method used, the average volume of hemolymph collected from the fruit fly is 30 nL from which amino acids are quantitated with capillary electrophoresis. The hemolymph was analyzed for differences in amino acid and other small biological molecules from each type of sampling method. It is known that the composition of the hemolymph can change when stress is created on the animal’s system. There were statistical differences found between the different sampling methods.

To understand how the processing and derivitization of the hemolymph can affect the chemical content, the amount of cells were determined. The cells collected in the hemolymph could be lysed during the derivitization process, which would cause the amino acid content to change. Cell sorting was completed to find the amount of cells in a hemolymph sample. Cells were sedimented from hemolymph via centrifugation. The amino acids in cells separated hemolymph and non-separated hemolymph were analyzed for statistical differences. In all, this project will lead to a better understanding of hemolymph at equilibrium and changes caused by the disruption of the physiological homeostasis.

**Abstract Text**

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Peptide substrate reporters are short peptides that act as readily-detected substrates for enzyme assays. The unmodified form of a fluorescent peptide substrate is separated from enzymatic products using capillary or microchip electrophoresis to report the activity of an enzyme of interest. Development of these reporters is challenging and often time-consuming because they must be optimized for stability and specificity, and separation conditions that resolve all possible enzymatic products must be identified. Despite the effort involved in developing substrate reporters, most are applied only to a handful of applications in a few types of cells. To assess the breadth of applications for these reporters, we are adapting a peptide substrate reporter for protein kinase B (PKB) that was developed in human cell lines for use in the social amoeba [i] Dictyostelium discoideum [/i]. While [i] D. discoideum [/i] expresses a PKB homolog, our results demonstrate that processing of a reporter may be quite different between species, even in a well-conserved system. We observe important differences in degradation of the reporter and the temporal dynamics of its phosphorylation in [i] D. discoideum [/i] compared to human cells. Additionally, we are exploring how the complexity of the experimental system affects reporter metabolism; reporter molecules are processed differently in solution with purified kinase, in lysates, and in intact cells. Finally, the method of loading the reporter into cells affects its localization and therefore its enzymatic processing. This presentation will discuss how these considerations affect novel applications of peptide substrate reporters.

Keywords: Bioanalytical, Capillary Electrophoresis, Enzyme Assays, Peptides
Application Code: Bioanalytical
Methodology Code: Capillary Electrophoresis
We show that G protein-coupled opsin family vision receptors (opsins), phototropin and cryptochrome based optical triggers are a powerful resource for controlling signaling in single cells with subcellular resolution. We mine the large and diverse opsin family to identify members with desired spectral and signaling properties to be developed as cellular signaling switches. By expressing these optical triggers and fluorescence molecular sensors, we show that distinct wavelengths of light can be used to control single cell signaling and capture response dynamics together. Using the confined activation of one such receptor; blue opsin, we direct migration of a variety of cells and selectively initiate-extend neurites from neuronal precursors. Imaging responses during this optically controlled process allows next generation mapping and analysis of signaling pathways in living cells. Using this approach, we have identified a group of G proteins that universally govern migratory and growth responses which are implicated in pathological conditions such as cancer and heart diseases. Similarly, we have identified an inhibitory signal propagation from the active site to the rest of the cell, allowing cells to achieve asymmetric behaviors in response to external stimuli. Additionally, using optical control of signaling and concurrent molecular imaging, we map the flow of information from the stimulus onset to the GPCR for the first time, G protein and downstream effector activation as well as the reverse of these processes after stimulus termination. In conclusion, our subcellular and single cell optical control and mapping of signaling can have wide applicability in many disciplines experimentally as well as therapeutically.

Keywords: Bioanalytical, Biosensors, Fluorescence, Imaging
Application Code: Bioanalytical
Methodology Code: Fluorescence/Luminescence
Bioanalytical - MS, GC/MS, and LC/MS

Metabolic Profiling Along the Segmentally Stratified Rat Colon

The large intestine is vitally important to human health. Also referred to as the “gut microbiome”, the large intestine contains more than 800 species of bacteria that help harvest energy, synthesize vitamins, and shape the immune system. Though it is critically important for human health and has been implicated in many diseases including obesity, diabetes, colorectal cancer, allergies, autism, and Crohn’s disease, the mechanism of metabolic communication between the gut microbiome and its human host remains poorly understood. NMR, GC-MS, and IC are used to identify and quantify major metabolites in extracts of fecal material isolated from the rat ileum, cecum, proximal, mid, and distal colon. These metabolites include short chain fatty acids that are the preferred energy source for colonocyte, amino acids, polyamines, bile acids, choline metabolites, phenolic compounds, and ions. This study will provide a wealth of new information on the metabolome, its stratification along the colon, and the assortment of metabolites available for absorptive transporters within each segment.

Keywords: GC-MS, Ion Chromatography, Metabolomics, Metabonomics

Application Code: Bioanalytical

Methodology Code: Gas Chromatography/Mass Spectrometry
We present the development of an acoustic droplet ejection (ADE) platform for label-free, high-throughput mass spectrometry (MS), capable of processing up to 3 samples per second. A standard Labcyte Echo[registered] ADE instrument has been modified to eject a spray of charged droplets. The droplets are desolvated in a transfer interface and the resulting ions are detected by a Waters MS instrument. This mode of ion generation is similar to ESI, but is performed directly from a 384-well sample plate, which allows for minimal sample consumption and real-time assay development.

A new modular transfer interface allows us to test a variety of parameters including droplet collection geometry, sheath flow, and desolvation temperature. We have also examined the effects of the sample plate profile and ejection acoustics. These new developments and advances greatly increase the platform’s sensitivity and reliability, allowing it to quantitate analytes at low concentrations in biological assays.

The Echo-MS has demonstrated the ability to support high-throughput analyses of biologically relevant drug assays. A biochemical screen of more than 300,000 compounds has been completed, was in concordance with traditional HPLC-MS, and used to inform a cell-based drug-targeting workflow. Several kinase assays have been converted from existing assay technologies to utilize the Echo-MS end point. Integration with automation capable of supporting a full file (1.8 million) compound collection screen has driven development of solutions to facilitate high volume analysis of spectral data and generation of output files that can seamlessly integrate with commercially available data analysis packages such as GeneData Screener[registered].

Keywords: Bioanalytical, High Throughput Chemical Analysis, Mass Spectrometry, Sample Introduction
Application Code: Bioanalytical
Methodology Code: Mass Spectrometry
# Bioanalytical - MS, GC/MS, and LC/MS

## Abstract

**Title:** 12-Time Point Proteomics of *Xenopus Laevis* Allows for Broad Understanding of Proteomic Expression File Emerging from a Mature Oocyte to Late Neurala Stage Embryo Quantifying More than 6,100 Protein Profiles

Quantitative proteomics for *Xenopus laevis*, was first reported in 2014 as a new tool in developmental biology research. My study examines proteomic and phosphoproteomic changes occurring during early development in the African clawed frog (*Xenopus laevis*), an important model organism for vertebrate development. I study changes in protein expression starting with the mature oocyte and progressing through the neurula stage (stage 22), by observing 12-distinct time points in development. Phosphorylation regulation during the first seven time points was analyzed and clustered alongside well-documented kinase activity, for the first time generating kinase profiles associated with important fertilization mechanisms. I have identified 6,339 quantifiable proteins and more than 3,900 phosphorylation sites in biological and technical duplicates. This study provides a solid baseline of proteins and phosphorylation sites essential for fertilization and early development. In particular, I have identified many proteins with distinct expression kinetics, and additional kinases of unknown function, which opens doors for further studies in early vertebrate development.

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

**Application Code:** Bioanalytical

**Methodology Code:** Liquid Chromatography/Mass Spectrometry

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**Room:** W175c

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It is often difficult to characterize or monitor biological cultures without impairment of cell growth or destruction of the whole sample. Non-invasive trace analysis of volatile organic compounds (VOCs) emitted from biological cultures could offer a solution.

We designed hermetically closed Teflon boxes with customized port connections for micro extraction devices. Depending on cell type and number the inner air volume of the boxes could be adjusted by means Teflon blocks. Preconcentration of volatile substances was realized through bidirectional needle trap micro extraction (NTME). Substances were separated by means of gas chromatography (GC), identified and quantified by means of mass spectrometry. To detect differences between media and media with cultures two identical boxes for parallel measurement were used. Heatmaps were used to visualize differences within the large amount of data.

Dilution-free sampling by means of NTME resulted in RSDs < 5%. Concentrations of VOCs were determined in the range of 1 ppb – 10ppb. Differences between cultures and media were most pronounced for oxygen and sulphur containing compounds. Media emitted significantly higher concentrations of hydrocarbons than cultures.

Air tight Teflon boxes with customized sampling ports enabled reproducible and reliable sampling from bacterial or cell culture headspace. Due to low methodological variations provided by the improved setup emissions from cell cultures could clearly be separated from those from blank media and ambient air. As even small variations in VOC concentrations can be assessed, VOC analysis could be used for destruction-free monitoring of culture growth.

Keywords: Bioanalytical, GC-MS, Sampling, Volatile Organic Compounds
Application Code: Bioanalytical
Methodology Code: Gas Chromatography/Mass Spectrometry
One of the main threats to the achievements of modern medicine is antimicrobial resistance. To find alternative methods for treatment of bacterial infections is essential to reduce the amounts of antibiotics used, and phototherapy has been suggested as one. Porphyrins, oxidation products of intermediates in the heme biosynthesis, are produced endogenously in most living organisms. These porphyrins have good photosensitizing properties and by illuminating bacteria with harmless blue light, an antimicrobial effect can be achieved. The porphyrin content in pathogens is however sparingly investigated, and when it has been done it is without chromatographic separation and/or with unspecific detection methods as UV-VIS/fluorescence.

We have developed a method for the extraction, clean up, and analysis of porphyrins in oral bacteria with HPLC-MS/MS. The porphyrin content in two pathogens associated with severe periodontitis has been determined using the evaluated method. Several potential photosensitizing porphyrins have been identified and quantified for the first time in these bacteria. By analyzing the porphyrin content in bacteria during different culture conditions, a large variation in the porphyrin profile could be seen, depending on time of culturing, passaging and agar composition. These results indicates that some bacteria produce porphyrins endogenously, and phototherapy has potential to be a novel tool to include in daily oral care for prevention of oral diseases as well as increase caries preventive aspect when incorporated into a traditional toothbrush.

Keywords: Bioanalytical, Chromatography, Liquid Chromatography/Mass Spectroscopy, Tandem Mass Spec
Application Code: Bioanalytical
Methodology Code: Liquid Chromatography/Mass Spectrometry
Aquaponics is an integrated technique that combines fish and plant production simultaneously. This systems only functions efficiently when nitrifying bacteria is present as a biofilter between the fish and plant components. The bacteria convert ammonia, a harmful waste product of fish digestion, to nitrates that are the preferred nitrogen source in plant metabolism. The lack of nitrogen buildup allows the water to be reused without the need for constant water exchange conserving more freshwater compared to typical aquaculture methods. Accumulation of total organic matter and toxic metals can cause oxidative stress for fish compromising growth and health. Selenium (Se) is a known antioxidant and many studies have supported its role in mediating oxidative stress, yet its use as an additive in fish feed is currently unregulated in the US.

The aim of this research was to supplement aquaponics systems with various concentrations (1, 2, 4 ppm) of Se to evaluate accumulation and metabolism of Se within the closed systems using ICP-MS and HPLC. Se accumulation followed the supplementation level in both fish and plants. Whole fish and edible muscle showed Se accumulation of approximately 1.5 ppm in the 1 ppm Se system and above 5 ppm for the 4 ppm Se supplemented system. Selenomethionine was found to be the major Se species in fish muscle using reverse-phased HPLC-ICP-QQQ with some being converted to selenocysteine. Plants showed the most Se accumulation in the roots (ppm level) for both basil and pinto beans with leaves and stems being much lower (ppb level). A Se mass balance of each system will be presented in addition to the Se species present in all foodstuffs.

Many aquaculture feeds also contain rare earth elements (REE) such as gadolinium, neodymium and samarium. The doubly charged species of these REE can cause false positives on potentially toxic elements such as selenium and arsenic. The benefits of ICP-QQQ reaction cell technology will be also be presented.

**Keywords:** Environmental, Food Safety, ICP-MS, Liquid Chromatography/Mass Spectroscopy

**Application Code:** Bioanalytical

**Methodology Code:** Liquid Chromatography/Mass Spectrometry
Bioanalytical - MS, GC/MS, and LC/MS

The Impact of Glyphosate on the Artemia Metabolome Determined Using 1H NMR and GC-MS

This study follows an environmental metabolomics approach to monitor the metabolic impact of glyphosate on Artemia franciscana (brine shrimp). Environmental metabolomics is the study of the metabolic response of an organism to environmental stressors. In this work, 1H NMR and GC-MS are used to analyze the metabolite fingerprint of brine shrimp and elucidate the metabolic mode of action of glyphosate. A total of 32 endogenous Artemia metabolites were identified and quantified by NMR and GC-MS. Isopropylamine from the Roundup formulation was observed by NMR with a doublet (1.29 ppm) and multiplet (3.5 ppm) in Artemia exposed to Roundup. It was found that a 48 hr exposure to Roundup at levels below the LD50 (237 ppm) in 35 g/L saltwater caused statistically significant changes (p < 0.5) to many free amino acids, such as alanine and lysine, and small molecules, such as choline and betaine. Increased levels of formate may be a result of methanol oxidation due to oxidative stress. Decreased levels of tyrosine may indicate inhibition in metabolic pathways related to synthesis of aromatic amino acids. This work shows that the Roundup formulation of glyphosate has an impact on the metabolome of Artemia, affecting metabolites related to energy metabolism and aromatic amino acid synthesis.

Abstract Text

Keywords: Environmental, Gas Chromatography/Mass Spectrometry, Metabolomics, Metabonomics

Application Code: Bioanalytical

Methodology Code: Gas Chromatography/Mass Spectrometry
Abstract Text
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 ESI and consequently varying degrees of sensitivity. These limitations may prevent the detection of molecules present in low abundance in biological samples. This work undertakes a derivatization approach to improve electrospray by tagging multiple functional groups to boost metabolite sensitivity. By tagging most functional groups, adduct formation and in-source fragmentation is dramatically diminished. This project is to use two distinct tags to label hydroxyl, amine, carboxyl, phosphoryl, and thiol groups on various metabolites and improve sensitivity in ESI-MS. The derivatization and separation approach offer advantages of rapid analysis of metabolites. This work will make the simultaneous detection of a diverse group of metabolites possible, leading to a more complete picture of the metabolic system of interest.

Keywords: Amino Acids, Liquid Chromatography/Mass Spectroscopy
Application Code: Bioanalytical
Methodology Code: Liquid Chromatography/Mass Spectrometry
Incomplete protein digestion into large peptides containing several missed-cleavage sites can increase sequence coverage in mass spectrometry (MS) analyses and help to identify the most protease-accessible regions of a protein. We control the extent of proteolysis by varying the protein-solution flow rate through membranes containing immobilized pepsin or trypsin. The platforms can include filters connected to syringes, membranes at the end of pipette tips, and spin membranes. Faster flow rates through a membrane lead to less cleavage and, hence, larger peptides. In the case of antibody digestion, fast flow and subsequent infusion electrospray ionization (ESI) MS lead to 100% peptide coverage and identification of post-translational modifications. Peptic digestion is particularly convenient because it occurs in MS-compatible acid solutions and avoids the need for cysteine alkylation. MS/MS analysis with several fragmentation methods gives 100% antibody light-chain sequence coverage. For de novo sequencing, we are exploring the use of multiple flow rates through the membrane to give small peptides whose masses sum to those of larger ones. Combined with the overlap of a few peptides, the relationships between peptide masses enable their arrangement for sequencing. Very rapid digestion of proteins induces cleavage only at the most accessible, labile sites. For example, digestion of cytochrome c in trypsin-containing membranes yields primary cleavage after a single lysine to give two dominant large peptides that cover the entire protein sequence. Changes in cleavage patterns during rapid digestion may identify alterations in protein conformations.
Bioanalytical Application of Mass Spectrometry

Expanding the Capabilities of Microscopy-Guided MALDI MS Profiling to Enable Analysis of Biochemically and Structurally Heterogeneous Biological Samples Ranging from Individual Neurons to Bacterial Colonies

Cellular heterogeneity is a vital property of multicellular biological systems and is responsible for homeostasis and diseased states. However, small sample volumes and large populations make single cell measurements difficult. We have recently developed a microscopy-guided MALDI-MS profiling method, which allows automatic targeting of cells for mass spectral analysis. The approach utilizes a fluorescent nuclear marker, Hoechst 33342, to localize dispersed cells. While the fluorescence images facilitate cell targeting, additional fluorescent markers and reporters can further stratify cell populations prior to MALDI-MS profile acquisition. Here, we present the latest version of our image analysis software for MALDI-MS profiling that further leverages biologically-relevant information contained within whole-slide images. Once cells are located, the cellular population can be partitioned based on size, circularity, or fluorescence intensity across multiple channels to incorporate specific fluorescent markers and reporters. Users select appropriate thresholds based on real-time interaction with images and histograms displaying the population distributions of the parameters. Filtering provides a rapid method to identify and select subpopulations that require additional MALDI-MS analysis. Examples including targeting larger, rare neurons surrounded by smaller, readily-abundant glia or astrocytes that retain certain endogenous or exogenous chemical markers, such as sulforhodamine. Finally, the software is easily adapted to systems beyond single cells, such as E. coli colonies. Direct, automated MALDI-MS analysis of bacterial colonies is difficult because the optimal matrix-to-analyte mixture occurs around the exterior of a bacterial colony. Therefore, we have developed a sampling method that accounts for the varied colony sizes and directs acquisition around the circumference of the colony. Several thousands of colonies are analyzed in a few hours with minimal user input.

Keywords: Bioanalytical, Imaging, Mass Spectrometry, Software
Application Code: Bioanalytical
Methodology Code: Mass Spectrometry
Endogenous neuropeptides show great promise as potential therapeutics due to their high potency and exquisite specificity, however they are limited by their vulnerability to enzymatic degradation and poor blood-brain barrier (BBB) penetration. Glycosylation can improve stability and BBB penetration of the resultant glycopeptides, but we need in vivo and in vitro methods for the quantification of glycopeptides. Quantification can be achieved using MS2 and MS3 analysis of specific peptide fragments. With glycopeptides, the sugar moiety is typically cleaved in the first fragmentation step with high efficiency, leading to a minimal loss of signal. In the second fragmentation step, the peptides fragment along the classical ABC/XYZ pathways. Use of MS3 allows for highly selective detection in a complex matrix, and the efficient fragmentation pathways for glycopeptides leads to increased sensitivity relative to the native unglycosylated peptides, improving limits of detection from hundreds of attomoles of material down to the single attomole level. Herein we present the quantification scheme for in vivo and in vitro degradation studies of Angiotensin 1-7 and PACAP/VIP derivatives needed to advance stable glycopeptide drug candidates that can penetrate the BBB.

**Keywords:** Liquid Chromatography/Mass Spectroscopy, Mass Spectrometry, Peptides, Tandem Mass Spec

**Application Code:** Bioanalytical

**Methodology Code:** Mass Spectrometry
For a patient with metastatic 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. Here, a novel [i]in vitro[/i] platform is used to assess the treatment of 3-dimensional colon cancer cell cultures, or spheroids, with combination chemotherapies.

Colon carcinoma HCT-116 cells are cultured with heavy or light SILAC media and grown into multicellular tumor spheroids. These spheroids are then dosed 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, the spheroids are harvested for quantitative proteomic profiling to examine the effects of the combination chemotherapy on the colon cancer cells. Spheroids are also assessed for cell viability, analyzed for drug concentration via LC-MS/MS and imaged for proteins and small molecules with MALDI-Imaging Mass Spectrometry. This innovative dosing device, along with the use of SILAC, gives a robust platform that can have a transformative impact on the pre-clinical evaluation of drug candidates. This system is high throughput, cost effective, and available to examine novel drugs and drug combinations, which can help to bring effective chemotherapies to market sooner.

Keywords: Bioanalytical, Drugs, Mass Spectrometry, Proteomics
Application Code: Bioanalytical
Methodology Code: Mass Spectrometry
The goal of this research is to sample large molecules at sub-micrometer spatial resolution for mass spectrometry proteomics and next generation sequencing genomics analyses. Atomic force microscope (AFM) tip-enhanced laser ablation is used to remove material from sub-micrometer region of a cell or tissue sample. The material is captured for genomic analysis or transferred to an ambient ionization source for on-line mass spectrometry. Cell and tissue samples are deposited a glass cover slip mounted on a circular metallic disc and ablated from 500 nm and 1 µm diameter spots. A pulsed visible wavelength laser is focused onto a metal-coated AFM tip for tip-enhanced ablation. The ablated material is sampled into a high-efficiency nanoelectrospray ion source where the ablated material is post-ionized for mass spectrometry. The system has been demonstrated for soft ablation of material for mass spectrometry analysis up to the size of small proteins and ablation of lipids from small regions of tissue samples. Plasmid DNA up to 3,000 base pairs has been successfully transferred and amplified with polymerase chain reaction (PCR) experiments. Ongoing work is aimed at developing this technology in two versions: 1) on-line electrospray post-ionization mass spectrometry with an ultra-low flow electrospray merged ionization source to create a mass spectrometry system with sub-micrometer sampling resolution and mass range up to the size of small proteins; 2) multiplexed capture of DNA material into 96 well plate for genomics and epigenetic studies.

Keywords: Atomic Force Microscopy (AFM), Imaging, Laser, Mass Spectrometry
Application Code: Bioanalytical
Methodology Code: Mass Spectrometry
The automation of an analytical technique has several advantages, such as greater reproducibility, faster sample throughput, and reduced analyst time for both method development and routine analysis. Traditional sample-preparation/separation methods used for the determination of prohibited substances in biofluids are time-consuming, require extensive sample pre-treatment, and have high operative costs. This study presents a novel SPME-based technology, named Coated Blades Spray (CBS), that efficiently integrates high-throughput sampling/sample preparation (96-well SPME configuration) with direct introduction to mass spectrometry. CBS was successfully implemented for the fast screening and quantitation of twenty prohibited substances listed by the World Antidoping Agency in plasma and urine samples. The whole analytical protocol consists of the following steps: a. extraction of the analytes for 1 minute from a vessel containing the samples (i.e. 96-well plate); b. fast removal of matrix constituents potentially adhered to the coated surface by a quick wash on a 96-well containing clean-water (5s); and c. direct-mass spectrometry analysis (30s MRM event per CBS). Limits of quantitation obtained for all the substances were in the range between 0.25-10 ng mL⁻¹ in urine and between 0.25-25 ng mL⁻¹ in plasma. Given that the extractions are performed in a 96-well format, the total analysis time is considerably less than one minute per sample. In summary, this work demonstrates that CBS-MS is an advantageous configuration for the rapid, sensitive, and cheap monitoring of prohibited substances in different biofluids.

Keywords: Bioanalytical, Mass Spectrometry, SPME, Tandem Mass Spec

Application Code: Bioanalytical

Methodology Code: Mass Spectrometry
Microfluidic paper-based analytical devices (µPADs) are cost-efficient analytical tools that can provide high-throughput analyses of complex samples. Unfortunately, µPADs are currently limited in their detection sensitivity and the available modes of detection, motivating research into improving the detection capabilities of these platforms. One promising approach is the use of ion concentration polarization (ICP) to pre-concentrate analytes within paper substrates, allowing for greater detection sensitivity. To expand the utility of µPADs, a novel ICP-based pre-concentration platform was developed and combined with MS detection via a paperspray ionization (PSI) emitter. A cation selective membrane was produced within a paperspray emitter by applying a small volume of Nafion solution and allowing to air dry. The resulting ICP-PSI system was capable of preconcentrating anions within the volume of the PSI emitter prior to MS detection. The device is operated in one of two modes to achieve ion concentration. The operation of this system in “ion-enrichment” and “ion-depletion” modes will be discussed, and the relative analytical advantages of each mode will be outlined. ICP-PSI emitters were tested against non-ICP PSI devices by preconcentrating dynorphin neuropeptides in a complex sample matrix. ICP experiments showed improved relative quantitation of dynorphin peptides than non-ICP experiments, demonstrating the efficacy of ICP-PSI for high sensitivity MS detection from paper-based microfluidic devices. Future work towards integrating paper electrophoresis into the PSI emitter will also be described.
Bioanalytical Application of Mass Spectrometry

Open Port Probe as a Robust Interface for the Direct Coupling of Biocompatible Solid-Phase Microextraction Fibers to Atmospheric Pressure Ionization Mass Spectrometry

The hyphenation of Solid Phase Microextraction (SPME) devices and mass spectrometry (MS) instrumentation have shown in recent years its great potential to improve limits of quantitation, accelerate the analysis throughput, and diminish potential matrix effects when compared to direct matrix introduction to MS. In this work we present the open port probe (OPP) as a robust interface to couple biocompatible-SPME (Bio-SPME) fibers to MS systems with minimal alterations to the front-end while maintaining sensitivity, simplicity, speed, and throughput. As a proof-of-concept, it is demonstrated the quantitative determination of clenbuterol in human urine, and the simultaneous quantification of codeine and hydrocodone in plasma. LC separation step was circumvented by utilizing two different techniques to gain selectivity, MRM3 and differential mobility spectrometry (DMS). Total sample-preparation/analysis time does not exceed 3 min and sample volumes of 300 L were used. Despite the short extraction/desorption times used (2 min/5 s), limits of quantitation (LOQ, 0.1 ng/mL for clenbuterol and 1 ng/mL for codeine/hydrocodone) below the minimum required performance level (MRPL) set by the world anti-doping agency (WADA) were obtained with good accuracy (90 %) and linearity (R2>0.99) over the range evaluated (0.1-10 ng/mL for clenbuterol, and 1-500 ng/mL for codeine/hydrocodone). Our results demonstrated that Bio-SPME-OPP-MS efficiently integrates sampling/sample clean-up and atmospheric pressure ionization, making it an advantageous configuration for several bioanalytical applications including doping in sports and therapeutic drug monitoring.

Keywords: Bioanalytical, Clinical Chemistry, Mass Spectrometry, SPME
Application Code: Clinical/Toxicology
Methodology Code: Mass Spectrometry
Ion mobility spectrometry coupled to rapid gas-chromatographic pre-separation is used for medical applications since about one decade now. Exemplary studies were carried out using human breath analysis for determination of the level of anesthetics as well as for diagnosis in nephrology. Furthermore, identification of pathogen bacteria and fungi after only 24 hours of cultivation could be demonstrated successfully, thus enabling earlier specific antibiotic therapy.

Another field was the support during animal models by breath analysis, thus gathering significantly more information on the development of a particular disease and providing information about possible biomarkers. Examples are studies on ARDS in a pig model, a feasibility study during an asthma mouse model and recently Sepsis in rabbit model.

The presentation will provide information on recent applications and on the status of method and hardware development.
Throughout history, cannabis has been used as a panacea, an herbal remedy for nearly all medical concerns from simple headaches to severe seizures. Now that many states have legalized medical cannabis, it is important to have analytical methods to study the compounds that the patients will be ingesting or inhaling. Terpenes are a major class of compounds found in cannabis. They are volatile hydrocarbons responsible for the plant’s aroma. These compounds were found to be medically relevant, reportedly assisting the cannabinoids in their effects. The cannabinoids themselves have medical relevance, and while their true effects are unconfirmed, the cannabinoids are undeniably helpful in some cases of seizures and pain. Our lab focused especially on five cannabinoids: 9-tertahydrocannabinol (THC), cannabidiol (CBD), cannabinol (CBN), cannabigerol (CBG), and cannabichromene (CBC). With the increase in usage of cannabis for medical ailments, a method for analyzing these compounds is necessary for regulation. Terpenes and cannabinoids were analyzed using a GC-FID with several injection techniques, including liquid injection, SPME, and headspace.

Additional experiments were performed to assess cannabinoid variance. To take their dose, patients can smoke the plant material, as opposed to pills or extracted oils. If the cannabis is not homogenized before distribution, the patient could be underdosing or overdosing. To test this, we determined the concentrations of the five major cannabinoids in homogenized and non-homogenized cannabis samples through liquid injection on a GC-FID. We found that homogenized cannabis has a lower variance than unhomogenized samples of the same plant. However, neither case meets the FDA variance requirement of less than two percent, suggesting this method of distribution should not be allowed for medical purposes.
Sampling methods, i.e. microdialysis and low-flow push-pull perfusion, have proven indispensable for studying neurochemicals; however, the traditional methods are inherently limited by poor spatial and temporal resolution. Our aim is to develop analytical technologies for overcoming these problems and demonstrate their uses for monitoring neurochemicals in vivo.

To enhance spatial resolution, our group has used a microfabrication approach to develop miniaturized sampling probes [1,2]. These probes were at least 5-fold smaller comparing to the traditional probes. In this work, we have successfully fabricated the probes with small sampling areas and three microchannels within a probe. The probes have overall dimension of < 100 μm. Further, we have incorporated the microfabricated probes with segmented flow for improving temporal resolution. Flow segmentation can easily be conducted by coupling the probe to a commercial tee. In addition, we have developed different microfluidic devices that can feasibly be coupled to the probe for droplet formation, reagent addition, and droplet extraction, resulting in even more integrated and portable system. For analysis, microchip electrophoresis coupled to laser-induced fluorescence was specifically employed in rapid separation (< 4 s) and detection of several amino acid neurochemicals.

With the use of the described system, monitoring of multiple compounds with high spatial resolution (over 10-fold better than traditional methods) and high temporal resolution (10 s or better) can be achieved. Preliminary studies have also shown suitability for sampling from small brain regions in live rodent brain.

Reference:

Keywords: Bioanalytical, Lab-on-a-Chip/Microfluidics, Neurochemistry, Sampling

Application Code: Neurochemistry
Methodology Code: Microfluidics/Lab-on-a-Chip
A New All-Polymer Microfluidic Chip to Measure Neurochemical Release from Single Cells

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.
Point-of-care testing (POCT) with the advantages of speed, simplicity, and low cost, as well as no need for instrumentation is of great importance to improve healthcare, ensure environmental safety, and guarantee food quality. Among various POCT platforms, Paper-based Analytic Device (PAD) is highly attractive due to their desired features, such as low cost, portability, disposability, flexible flow control as well as easy fabrication and operation. Herein, we will present our recent progress on integration of target-responsive smart hydrogel with \textit{PAD} for POCT. The platform relies on target-responsive aptamer-crosslinked hydrogel for target recognition, and cascade enzymatic reactions for signal amplification, \textit{PADs} for visual quantitative readout. With the advantages of low cost, ease of operation, general applicability, and disposability, the hydrogel-\textit{PAD} holds great potential for portable detection of trace targets in environmental monitoring, security inspection, personalized healthcare, and clinical diagnostics.
Online tissue monitoring is crucial in clinical environments. However, there are many challenges associated with making measurements in unknown tissue where analyte concentrations and features of the sample matrix have not been established. Using microdialysis sampling coupled with online microfluidic biosensors can overcome some of these obstacles as analysis can be carried out away from the tissue (1,2). However, traditional integrated biosensors can be challenging to optimise and their use is limited to analytes with relatively simple detection mechanisms. A platform that allows methodical optimisation of the analysis system for a specific application would be highly beneficial.

We have developed a flexible microfluidic platform that consists of LabSmith computer-controlled syringe pumps and valves. In its simplest form this system can be configured to allow automatic calibration of integrated needle biosensors in a microfluidic chip. The calibration script can be programmed to repeat at regular intervals to track the sensitivity and stability of biosensors, which is vital for long-term monitoring.

This platform can be configured to carry out multi-point standard additions to quantify sensor performance in real tissue streams. Alternatively, it can be used to add in buffers to control the pH. As the platform allows complete control of low-volume flow streams, it also provides the flexibility to add in enzymes and cofactors to react with the substrate in flow. In this setup the reagent concentrations and flow rates can be optimised independent of the downstream electrode. This lends itself particularly well to analytes with multi-factorial detection mechanisms, which would otherwise be hard to measure and optimise.

Examples of the application of these systems to tissue viability monitoring in various clinical applications will be presented.

References:
The number of approved, new antibiotics continues to decrease each year. A primary reason is that bacteria often become resistant to an antibiotic soon after becoming commercially available, thus rendering such efforts financially unattractive. The CDC, NIH, and FDA have made it a goal to develop new antibiotics and diagnostic tools to better understand resistant bacteria. Here, we present a 3D printed, fluidic device that determines a bacterium’s susceptibility to antibiotics. The device’s layout is designed to mimic the antibiotic’s path through the body (which involves absorption into the blood, diffusion into the tissue, and excretion). The layout is also 96-well plate compatible. This method utilizes the release of ATP from growing bacteria in samples exposed to biological concentrations of antibiotics. The bacteria are grown for 2 hours to mid-logarithmic growth phase and then added into a compartment, representing tissue, followed by the diffusion of the antibiotic to desired concentration (5 mg/L for Levofloxacin) by continuous flow of antibiotic-containing physiological buffer. The ratio of ATP release to bacterial turbidity is measured at various time points to determine if the antibiotic is able to kill the bacteria. In a static experiment, a non-resistant strain shows a 100% or higher increase in the ATP to turbidity ratio 20 minutes from the time a bactericidal antibiotic was added when compared to the ratio when no antibiotic is present. A resistant strain should show no significant change in this ratio, thus representing a method to determine antibiotic resistance in less than one-half hour.

Keywords: Biotechnology, Lab-on-a-Chip/Microfluidics, Luminescence, Pharmaceutical

Application Code: Pharmaceutical
Methodology Code: Microfluidics/Lab-on-a-Chip
Abstract Text
Carboplatin is an effective anti-cancer drug which is most widely used for the treatment of a variety of malignancies. Carboplatin affects not only the cancer cell growth but also blocks the growth of healthy rapidly dividing cells, leading to many side effects. This study aims to fabricate a system to allow detection of the presence of the anti-cancer drug carboplatin in healthy tissue in real time during chemotherapy. Real-time measurement of the biochemical changes is most easily achieved using microdialysis as the sampling probes are FDA approved, but such probes typically produce dialysate for on-line analysis at 10-30 nl / sec. We have perused the development of microfluidic ‘circuits’ using LabSmith components that can give us precise fluid delivery, excellent temporal control of multiple liquid streams. Detection of carboplatin can be carried out by observing the effects of the drug on the differential pulse voltammetry of free purine bases using a novel carbon nanotube-epoxy composite electrode as compared to conventional electrodes, glassy carbon electrodes. Real-time carboplatin detection will be performed using a computer-controlled microfluidic platform. The methodology will be optimised in terms of the analysis time and of to allow repeated carboplatin measurement. Microdialysis sampling and our microfluidic platform are combined to give a proof of concept system for real-time carboplatin detection.

Keywords: Biomedical, Detection, Electrochemistry, Lab-on-a-Chip/Microfluidics
Application Code: Biomedical
Methodology Code: Microfluidics/Lab-on-a-Chip
Bio-sample preparation such as cell concentration adjustment is routinely implemented mainly using centrifuge which is extensively laborious (manually operated), time-consuming, suffering from severe cell loss and requiring experienced technician for reproducibility. In this work, we report a low-cost microfluidic device for rapid automated concentration of cells, which offers low cell loss and high profile of reproducibility. Our device was fabricated using standard soft photolithography and inertial forces were employed to focusing cells into tight streams in the channel center. Flexible outlet system was designed to exclude the cell-free medium after cell focusing and thus enhanced sample concentration. Our results have shown the highly linear correlation between outcome Hep G2 cell concentration and the resistance configuration of outlet system. For a given sample, precise outcome concentration can be achieved by proper selection of pre-defined outlet system. Our single channel device is capable of processing 5 ml cell suspension in less than 20 min as compared to 30 min using centrifuge. Repeated experiments have also shown less than 5% cell loss—a substantial improvement amid the average cell loss of 40% during centrifugation and resuspension which are completely eliminated in our flow through device. The low cell loss can be extraordinarily important when processing sample with scanty cell population. In conclusion, we have successfully demonstrated a simple microfluidic chip capable of automated adjustment of biosample concentration. In light of its low cell loss, rapid processing and easy integration into Lab-on-a-Chip system, we envision its expanding applications in bio-sample preparation.

**Keywords:** Biotechnology, Lab-on-a-Chip/Microfluidics, Sample Handling/Automation, Sample Preparation

**Application Code:** Biomedical

**Methodology Code:** Microfluidics/Lab-on-a-Chip
Portable High-Resolution 3D Printed Microfluidic Analyzer for Online Clinical Microdialysis Samples

Clinical microdialysis is a powerful tool for monitoring the metabolic state of tissue in a wide range of applications. Traditionally, dialysate is analysed off-line in discrete samples. This results in poor temporal resolution and causes the magnitude of dynamic changes to be diluted over the sample collection period. In contrast, continuous online microdialysis has been recently developed to overcome these issues[1]. Combined with microfluidics and online biosensing systems, it provides high time resolution suitable for resolving dynamic changes in metabolite levels.

We have developed a system in which the dialysate stream continuously flows into our newly engineered analyser. Our system consists of a high-resolution 3D printed microfluidic flow cell, which builds upon the first generation of our robust microfluidic manifold that attaches to a clinical microdialysis probe[2]. It incorporates removable needle type amperometric biosensors for glucose and lactate, which are connected to our newly designed miniaturised wireless potentiostat. The whole device will then be enclosed in a 3D printed casing to make it a truly portable device, allowing the analyser to be positioned closer to the tissue, improving the sensing resolution further still.

Optimisation of the 3D printed channel size and the resulting improvement in the sensor response time will be presented here together with the design of portable electronics. This truly portable lab-on-a-chip analyser has many potential applications. Proof-of-concept results will be shown for two clinical applications in which this technology would be particularly beneficial: monitoring transplant kidneys in transit where a portable system to provide time-critical information is essential and monitoring the injured human brain where a wearable system would allow better resolution of transient metabolite events.


Keywords: Biosensors, Electrochemistry, Lab-on-a-Chip/Microfluidics, Portable Instruments

Application Code: Biomedical

Methodology Code: Microfluidics/Lab-on-a-Chip
Microfluidics has led to advancements in portable analysis for point of care testing, environmental monitoring and Homeland Security measures. However, the high cost of development and fabrication of devices has limited the development to wealthy countries and well-funded laboratory settings. This has limited the development and the adoption of these devices in depressed economic areas which are often the target for Lab-on-a-chip devices due to poor infrastructure and limited resources. We present here a low cost fabrication technique for microfluidic molds using 3D printing technologies and advance microcontroller techniques. Not only can this technology be used for the development of new onsite analysis by resource limited government and industrial labs but its low cost and easy of fabrication make it ideal for undergraduate research and education by lowering the overhead cost of development. We have designed and built a spin-coater for photolithography using AutoCAD inventor and a 3D printer. We have then integrated a microcontroller to precisely control the spin rate and time for the coating of silicon wafers with photoresist in a user friendly package. We have calibrated the spin rate externally with a laser tachometer and then correlated feature height as measured by profilometer to accepted product literature values. We then patterned the photoresist with an inexpensive UV lamp system to develop the microfluidic pattern. All of this process takes places without the requirement to be in a cleanroom. We will demonstrate a proof of concept separation and analysis of common metal contaminates found in coal ash spills, Selenium, Cadmium, Lead, and Nickel using microfluidic devices fabricated from this process. We will outline these low costs alternatives to the fabrication of microfluidics based on open source support for both the 3D printed components and the code for the microcontroller to help facilitate further advancements in resource limited settings.

Keywords: Education, Environmental/Water, Instrumentation, Lab-on-a-Chip/Microfluidics
Application Code: Environmental
Methodology Code: Microfluidics/Lab-on-a-Chip
In the research of flow injection analysis, flexible polydimethylsiloxane (PDMS) based devices have been used and them simple optical aperture for vertical observation due to the low autofluorescence.[1,2] However, when more complicated optical structure tried to embedded horizontally, complicated optical blocking structure must be introduced to suppress the severe scattered stray light. [3]

Recently, we proposed the novel optical design in a two-layer structure which are the transparent PDMS layer and the black pigment dispersed PDMS layer. Boundary less absorbance layer can suppress the internal light scattering effectively. [4] In addition, the basic optical functions such as light refraction and filtering were implemented based on the molding with optical master parts and doping absorbing dye, respectively. These concept, termed as Silicone Optical Technology (SOT), were demonstrated as some applications. [5-7]

In this paper, we report print-like-manufacturing study of SOT using low melting-point metal Ga and PDMS. Ga/PDMS boundary [8] can proved metallic reflection in optical quality in liquid-state. Firstly, inject and form liquid-state Ga into the PDMS, then cool and solidify Ga by cooling. Next, cure PDMS in room temperature with solid-state Ga mold. Due to the surface tension difference, spherical and semi-plane surface has sufficient quality for optically use. Reflection grating and mirror structure are also studied.


Keywords: Flow Injection Analysis, Integrated Sensor Systems, Lab-on-a-Chip/Microfluidics
Application Code: Environmental
Methodology Code: Microfluidics/Lab-on-a-Chip
Polycyclic aromatic hydrocarbons (PAHs) are persistent organic pollutants which are a concern for the environment and human health because of their toxicity, potential long range transport and bioaccumulation. Conventional laboratory analysis implies important costs and labour, and may lead to low sample representativeness due to sampling, transport and storage prior to analysis. To date, no portable equipment enables in situ PAH determination with adequate selectivity and sensitivity.

In this context we report the development of a novel silicon/glass lab-on-a-chip for the extraction of organic pollutants such as PAHs from natural waters. The device consists in an optimized microfluidic chamber filled with micro pillars whose surfaces are functionalized with adequate chemical coating. PAHs pre-concentration recoveries after solvent desorption have been obtained by HPLC associated with fluorescence detection. Two coatings, PDMS (polydimethylsiloxane) and a novel nanoporous organosilicate thin layer, have been tested, the latter showing better results when one considers the full range of PAHs. A comparison with the commonly used SBSE (Stir Bar Sorptive Extraction) laboratory technique shows that equivalent extraction recoveries are obtained but in 50 times less time. Therefore these new extraction microchips appear as a good candidate for further development of field analysis systems.

This work was supported by the French National Research Agency (ANR) through Carnot funding.
A One-Step Surface Modification Method for Simple DNA Immobilization on Paper-Based Device and Its Application for DNA Detection

Glass slides have been widely employed for DNA immobilization in DNA microarray since past decades, whereas they are faced with limitations of complicated fabrication procedures, time-consuming modification, low probes density, and expensive instruments. In this work, we developed a simple one-step surface modification method to graft DNA codes on paper, avoiding redundant cross-linking reactions in conventional methods. The Cy3-labeled DNA capture probe was simply immobilized on the aminosilane-derivatized paper-based device via the ionic adsorption, after one-step surface modification with 3-aminopropyltrimethoxysilane (APTMS). FT-IR spectra, X-ray photoelectron spectroscopy (XPS) and fluorescence detection were employed to characterize the surface modification and the subsequent DNA immobilization. Incubation conditions of time and temperature were optimized, and the adsorption mechanism was studied systematically. Furthermore, this APTMS-modified paper-based device was successfully applied for the pathogen detection of Giardia lamblia. Compared with conventional methods using abundant cross-linking reactions, this method is simpler, faster and lower-cost, and will broaden the application of paper-based bioassays for point-of-care diagnosis in resource-poor settings.

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Keywords: Bioanalytical, Characterization, Immobilization, Lab-on-a-Chip/Microfluidics
Application Code: Bioanalytical
Methodology Code: Microfluidics/Lab-on-a-Chip
Microfluidic devices have begun to change the way cellular analysis is performed. They are capable of analyzing multiple analytes from single cell lysates at rates of up to 20 cells/min for periods of over an 1hr. These rates are sufficient to generate statistically significant results in reasonable time periods. One potential application for these devices is the rapid profiling of kinase, protease, and cytokine activities in tumors. The cells in tumors are heterogeneous due to the rapid mutation rates in the cells comprising and recruited to the tumor. Chemotherapy works best if the drug cocktail applied can be optimized for the different cellular mutations contained in the cells in the tumor. Such information cannot be obtained from a bulk analysis of the cells. It must be obtained via the analysis of individual cells.

We have developed a novel microfluidic device architecture that integrates an optical fiber into a microfluidic device to provide 3 excitation and 3 detection spots using only 1 excitation source and 1 detector. This was done by taking advantage of a tunneling mode available in multimode fibers. Being able to detect the lysis event and the completeness of the lysate injection is important as it allows one characterize how well a system is behaving, to monitor whether something begins to go wrong over the course of an analysis, and to identify analytes in a multicomponent separation based upon their absolute migration times. This device was used to determine kinase, protease, and cytokine activity in cells.

Keywords: Bioanalytical, Capillary Electrophoresis, Lab-on-a-Chip/Microfluidics
Application Code: Bioanalytical
Methodology Code: Microfluidics/Lab-on-a-Chip
Traumatic brain injury (TBI) is the third most leading cause of deaths in the USA. In addition, those who survive the primary impact of TBI can still suffer from severe secondary effects, such as cognitive disabilities and epileptic seizures. Unfortunately, the biochemical mechanism of the neurodegeneration of these secondary effects caused by the TBI is still not well understood. Purine compounds, including adenosine, inosine, and hypoxanthine, have been recognized as biomarkers for TBI. Monitoring the extracellular concentration changes of these biomarkers in the brain can be useful for better understanding the biochemistry of neurodegeneration after a TBI. In this study, the development of an on-line microdialysis-microchip electrophoresis (MD-ME) separation-based sensor with amperometric detection (AD) for monitoring of adenosine, inosine, hypoxanthine, and guanosine, in brain extracellular fluid is described.

Initially, the ME-EC device was optimized to achieve high separation efficiencies and low limits of detection (LOD). Separation and detection of the four biomarkers was accomplished in less than 90s with a baseline resolution using a PDMS chip with a carbon fiber (CF) working electrode in the near-end channel configuration. LODs of 5, 10, 10, and 33 [micro]M were achieved for hypoxanthine, adenosine, guanosine, and inosine, respectively. CF electrodes modified with graphene oxide and a dual-channel/dual-electrode microchip design for on-chip background subtraction are currently under investigation to further improve the LODs of the system. The ME-AD device with a CF electrode has previously been coupled to microdialysis sampling and will be used for on-line monitoring of the four biomarkers in rat brain.
Membrane proteins play pivotal roles in cellular processes ranging from membrane transport to cell-to-cell communication, and these proteins are the most common targets for pharmaceutical agents. Key to the study of membrane proteins is maintaining protein function in vitro. Isolating functional membrane proteins from cells is challenging because of low solubility in aqueous solutions, poor activity out of the native membrane environment, and limited expression in most cell culture systems. Soluble lipid bilayer systems, such as protein-lipid micelles and liposomes, act as a water-soluble and semi-native environment, which allows characterization of many membrane proteins. Nanodiscs, an alternative to liposomes and micelles for membrane protein analysis, are soluble, protein-stabilized phospholipid bilayers that provide a native-like environment to study membrane proteins with the added advantages of precise control of bilayer composition, stoichiometry, and size as well as access to both sides of the bilayer. We have developed a microfluidic device capable of functional membrane protein studies via on-chip Nanodisc assembly. The device incorporates membrane proteins into Nanodiscs starting from cell lysate, and purification of Nanodiscs is performed on-chip. Nanodisc assembly and purification can be completed in less than 1 h with minimal sample consumption (~1 ng of membrane protein). We have applied the device for functional studies of a diverse set of membrane protein classes, including cytochrome P450 enzymes and receptor tyrosine kinases.
Barriers formed by cellular tight junctions, such as those lining the brain, intestines, and vascular system, segregate biological systems and modulate the diffusive transport of biological and pharmaceutical molecules between these systems. The dysfunction of these barriers in disease states can be pathologically revealing. Increased barrier permeability is observed in human diseases of widely ranging etiology, from viral hemorrhagic fevers (VHF) to autism spectrum disorders (ASD). An in vitro barrier mimic allows the study of mechanisms affecting barrier permeability in a precisely controlled environment not possible with in vivo models. Here, microfabrication procedures were developed to produce a microfluidic platform in which endothelial cells were cultured to confluence to form a three-dimensional blood vessel mimic of vascular barriers. Soft lithography techniques were used to produce a polydimethylsiloxane (PDMS) support in which hydrogels were molded around a 60-μm diameter fiber template. Removal of the template left a cylindrical channel which became the support for adherent cell culture. Conventional microfluidic devices rarely incorporate hydrogels as substrate materials, thus new approaches were developed to achieve common microfluidic tasks, such as coupling the device with pressure driven flow from external pumps. These new approaches enabled the integration of a perfusion system to deliver shear flow similar to that experienced in vivo. Microfabrication procedures, cell culture protocols, and cellular imaging strategies will be described. Future directions for modeling VHF and ASD-like conditions in this system will also be discussed.

Keywords: Bioanalytical, Lab-on-a-Chip/Microfluidics, Method Development
Application Code: Bioanalytical
Methodology Code: Microfluidics/Lab-on-a-Chip
**Microfluidic Methods - Bioanalytical Applications**

**Electrokinetically Operated Integrated Microfluidic Platform for Immunoaffinity Extraction and Electrophoresis of Preterm Birth Biomarkers**

Preterm Birth (PTB), a birth prior to 37 weeks of pregnancy, affects more than 500,000 children every year in the United States. It is also the most common complication in pregnancy and is the leading cause of neonatal deaths and newborn illnesses. There is a significant need for a rapid and inexpensive diagnosis method which can predict PTBs at a stage where therapeutic interventions are still possible. Esplin at al. [1] recently characterized a biomarker panel of 6 proteins and 3 peptides, the analysis of which can predict a PTB with 87% sensitivity and 81% specificity. Our aim is to develop an electrokinetically operated integrated microfluidic platform that can analyze these biomarkers and provide an early diagnosis of PTBs. We have previously developed and reported on-chip fluorescent labeling, preconcentration, and electrophoresis devices for PTB biomarker analysis [2, 3]. In this work, we have developed affinity monoliths with immobilized antibodies to specifically capture and selectively elute PTB biomarkers. Using these devices we have demonstrated immunoaffinity extraction of ferritin, a PTB biomarker, from solutions with low nM concentration. We are also integrating this approach with microchip electrophoresis to simultaneously capture, preconcentrate, selectively elute and separate multiple PTB biomarkers. We believe this work will provide significant progress towards the eventual development of an integrated platform for early and cost-effective diagnosis of PTBs.

**Acknowledgements:**
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**References:**

**Keywords:** Bioanalytical, Electrophoresis, Lab-on-a-Chip/Microfluidics, Solid Phase Extraction

**Application Code:** Bioanalytical

**Methodology Code:** Microfluidics/Lab-on-a-Chip
Acute lymphocytic leukemia (ALL) is most often found in children and about 6000 cases are reported each year in the United States. The peripheral blood lymphoblast percentage is an important index for ALL diagnosis and prognosis. We describe a microfluidic system that isolates and enumerates peripheral blood lymphoblasts in a microfluidic affinity region, and then the captured lymphoblasts are recovered for the downstream analysis. Well characterized CCRF-CEM cells (human T cell lymphoblast-like cell line) are spiked into human blood sample with different initial percentages (5%-30%). The mixed sample is injected into an herringbone affinity surface that is coated with a selected monoclonal antibody, with a continuous flow at an optimized flow rate to retain the CCRF-CEM cells while elute the background blood cells. Different fluorescent signals are detected to identify and enumerate captured CCRF-CEM and blood cells, respectively. With a low spiked CCRF-CEM ratio sample (<30%), a capture purity of 85%-95% is achieved. This inexpensive and effective method will potentially be applied in diagnosis and prognosis of ALL in point-of-care.
We report the new separation method of the microdroplets’ contents using spontaneous emulsification aimed at single cell analysis. Recently, droplet microfluidics involving aqueous microdroplets formed in microfluidic devices have been paid much attention in the fields of biochemical and chemical analyses. Since various substances such as molecules and cells can be isolated in each microdroplet, the microdroplets is used as small chemical containers. In addition, the microfluidic devices enable to manipulate the microdroplets at the rate of over 1000 droplets per second. Therefore, the droplet microfluidics is expected to have much potential on the application to high-throughput analyses such as single cell assays. However, the lack of the separation method for large water-soluble solutes, such as protein, in microdroplets has limited the applicability of the droplet microfluidics to the biochemical analyses.

In order to overcome this difficulty, we conceived to use the combination of the inversed micelle extraction and spontaneous emulsification at the interface of the microdroplets. When an aqueous microdroplet (1 nL size) was exposed to the hexadecane containing 66 mM Span 80, a nonionic surfactant, and some anionic surfactants, nanodroplets were formed at the interface of the microdroplets as the result of the spontaneous emulsification. During spontaneous emulsification, it was found that several kinds of proteins such as lysozyme and BSA can be separated from the microdroplets to the nanodroplets while nanoparticles larger than 50 nm were not. By utilizing this phenomenon, we will demonstrate biochemical assays in single microdroplet aimed at single cell analyses.

In the presentation, the control of the selectivity of this separation method and the application of this method to biochemical analyses will be presented.

Keywords: Extraction, Isolation/Purification, Lab-on-a-Chip/Microfluidics, Surfactants
Application Code: Bioanalytical
Methodology Code: Microfluidics/Lab-on-a-Chip
The need for field portable devices that could respond to the today’s requirements for low cost and rapid detection with on-site measurement capabilities continues to grow. This presentation will discuss development, scalable manufacturing, analytical characterization and deployment of portable biosensors that incorporate functional redox active nanoparticles, and their applications for environmental, clinical and food monitoring. A unique feature of these devices is the built-in detection mechanism with all the sensing components needed for analysis deposited onto the sensing platform. The modified surface integrates recognition, signal amplification and detection capabilities and can function as an all-in-one bioanalytical device. The sensors can be mass produced by printing which enables roll-to-roll manufacturing using a low cost, versatile and controllable process. Recent work focusing on the development of nanoparticle-based colorimetric tests and printed enzyme-based paper sensors for point-of-care diagnosis, food monitoring and personal exposure assessment as well as sensors for detection of engineered nanoparticles will be discussed, with examples of applications. The sensors have been interfaced with portable databases and user-friendly signal transduction methods, and have demonstrated excellent analytical performance when used in the field.
Recent Developments in Portable Instruments

Novel Non-Radioactive Ion Source for Atmospheric Pressure Ionization (API)

For many spectroscopic methods the ion source is an irreplaceable part. In particular for handheld instruments radioactive ion sources (e.g. Ni63) were common. Because of legal restrictions the usage of non-radioactive ion sources becomes more and more important. In a research project named BRANDI a new non-radioactive ion source was developed. One aim of the project was to engineer a micro electron gun for the use with a commercial available ion mobility spectrometer (IMS). The developed ion source should ionize in a similar way a radioactive ion sources (e.g. Ni63) does. For that a micro electron gun was constructed that accelerates electrons in vacuum. After trespassing a membrane the electrons have enough energy to initialize the well described two stage ionization process under atmospheric pressure conditions.

In the recent talk we will present the first results with the non-radioactive micro electron gun ion source for use with a IMS. For the application it is important to detect positive as well as negative ions. We will show the evaluation of the non-radioactive ion source based on the nature of the positive and negative reactant ions as well as on selected target chemicals, e.g. chemical warfare agent (CWA) stimulants and toxic industrial chemicals (TIC).

For further characterization we will compare the micro electron gun ion source with corona discharge ionization techniques. We will discussed similarities and differences based on conducted measurements and will show advantages and disadvantages of the micro electron gun ion source.

Keywords: Portable Instruments, Spectrometer, Spectroscopy, Volatile Organic Compounds
Application Code: Safety
Methodology Code: Portable Instruments
Recent Developments in Portable Instruments

Hand-Portable Nanoflow Liquid Chromatography System

Although there has been increasing interest in portable gas chromatography (GC), there has not been as much interest in portable liquid chromatography (LC). This is despite the demonstrated usefulness of portable GC and the added advantages that LC would provide for chemical detection in the field. Portable LC has unique challenges that make development of a system more difficult.

In this work, we linked two syringe pumps to form a binary gradient module with a 6000 psi pressure rating. The internal volume of these syringes is 250 [micro]L, so at a typical flow rate of 500 nL/min, more than 8 h of analyses are possible before a refill is needed, making solvent reservoirs unnecessary for field work. In addition, the extremely low flow rates make waste collection and disposal very simple. Multiple binary gradient modules can be combined together to provide needed selectivity for target analyte detection. Each dual pumping module is compact, measuring ~2” x 3” x 8”, weighing less than 1.5 lb. and consuming less than 10 W. A battery pack can be used to run the system, which provides ~8 h of run time and weighs ~1 lb.

In addition to the compact pumping system, we designed a compact dual-wavelength UV absorbance detector for on-column detection. This configuration, combined with novel optics, provides performance that is ~100x better than other commercial UV-absorption detectors. The addition of an injector and a 150 [micro]m i.d. packed capillary column provide a high-performance system that is light, portable and able to operate using battery power for field analysis.

Keywords: Drugs, HPLC, Portable Instruments
Application Code: Clinical/Toxicology
Methodology Code: Portable Instruments
Recent Developments in Portable Instruments

Dopant-Assisted Positive Photoionization Ion Mobility Spectrometry for On-Site Detection of Peroxide Explosives

Peroxide explosives, such as triacetone triperoxide (TATP) and hexamethylene trioxide diamine (HMTD), have been used many times in the improvised explosive devices for terrorist attacks in the past decade, due to their easy synthesis from easy-to-obtain materials. Thus, it is a very urgent need to develop rapid and on-site device and method for TATP and HMTD. Herein, a stand-alone dopant-assisted positive photoionization ion mobility spectrometry (DAPP-IMS) couple with time-resolved thermal desorption introduction for rapid and sensitive detection of TATP and HMTD will be presented. Acetone was chosen as the optimal dopant to obtain higher sensitivity and better separation between reactant ion peaks and product ion peaks. Owing to the different volatility of the explosives, the time-resolved thermal desorption method for TATP and HMTD was established by thermally desorbed of the analytes on the sampling swab and carried into the ionization region dynamically. As a result, the detection of TATP and HMTD down to 23.3 and 0.2 ng, respectively, in complex matrices, such as white solids, soft drinks, and cosmetics, were achieved within 10 s. Furthermore, with the combination of the fast response thermal desorber (within 0.8 s) and the quick data acquisition software to DAPP-IMS, two-dimensional data related to drift time and desorption time was obtained for TATP and HMTD, which is beneficial for their identification in complex matrices. It is demonstrated that the DAAP-IMS is a powerful device to identify the peroxide explosives in complex matrices.

Keywords: Forensics, High Throughput Chemical Analysis, Portable Instruments, Trace Analysis
Application Code: Homeland Security/Forensics
Methodology Code: Portable Instruments
The presence of harmful chemicals inside cargo containers is a recognized occupational safety problem among harbor workers. Cargo containers can contain fumigants for biological security reasons and other harmful compounds due to the off-gassing of chemicals used in the manufacturing or packaging. The current practice is to ventilate the cargo before unpacking to avoid the risks. However, even containers declared as ventilated may still have a high concentration of hazardous chemicals inside when opened. The only way to be sure that a container is safe is to measure the chemicals inside it. Real-time monitoring techniques used in practice are insufficient as they do not detect most of the harmful chemicals [1,2] and the detailed analysis of several chemicals requires sophisticated and expensive measurements.

We address this problem with hand-held laser-based photoacoustic detection. A widely tunable matchbox-sized external cavity quantum cascade laser (EC-QCL) source is used to scan over mid-infrared region and an ultra-sensitive patented cantilever sensor is used to record the photoacoustic signal. The whole system including electronics and batteries can be fitted in hand-held size and can achieve sub/low-ppb detection limits depending on the target gas. Multi-gas measurement capability with ppb level detection limits in real cargo container environments is demonstrated.

This research project is co-funded by the Horizon 2020 programme of the European Union.

Ion mobility spectrometers (IMS) are widely used in different applications. The ambient pressure operation of these instruments yields the dependency on several environmental parameters. The influence on the mobility of some parameters, e.g. temperature and pressure is already accounted for by compensation measures. In this talk we will point out the significant influence of humidity on ion mobility measurements. Up to now there is no established method for humidity compensation.

In the first part of the talk we will focus on reliable methods for humidity measurement in the lower ppm range. We will compare the performance of two humidity sensors (Michell Instruments, EA2-TX-100 and Moisture trace, MT1000 based on Phosphorpentoxid (P2O5)) at the boundary operating range. The applicability of both sensors for use in an IMS as permanent sensors for humidity compensation issues will be discussed. We will present numerical simulations of the humidity distribution in a closed IMS drift gas loop, which indicate possible solutions for the control of humidity levels.

Furthermore we will show experimental results that confirm the numerical simulations and show the enormous influence of humidity on IMS measurements. We will point out the importance of an accurate instrument setup. We will show the comparison of theoretically predicted humidity levels deduce from clustering theory with the actual measurements of both humidity sensors. Finally we will present a novel method for control of the humidity level in an IMS. With this method a compensation of different humidity levels is feasible and more reliable IMS measurements are possible.

**Keywords:** Detection, Detector, Instrumentation

**Application Code:** Homeland Security/Forensics

**Methodology Code:** Portable Instruments
Recent Developments in Portable Instruments

Portable Gas Analyzer for Continuous Monitoring of Hydrogen Sulfide in Gas Streams

Design, construction, optimization and application of low-cost portable analyzer for real time monitoring of hydrogen sulfide in gas stream are described. The analyzer is based on our patented (US Patent number: 8,709,820) detection scheme of hydrogen sulfide. This is based on absorbing hydrogen sulfide into alkaline solution and then oxidizing the absorbed sulfide ions with hydrogen peroxide. The reaction is significantly exothermic and the temperature rise of the reaction medium proved to be a very reliable analytical signal for the quantification of hydrogen sulfide. The present analyzer offered significant improvements over our previous report (Sensors and Actuators B 2012, 162, 377-383) which include: (i) improved detector design, (ii) elimination of the diffusion scrubber, (iii) compact and portable design, (iv) faster response time (~1.5 min) compared to ~ 8 min in the previous report, and (v) more sensitive detection. The analyzer construction contained battery operated two dual-channel peristaltic pumps, 4-channel Thermocouple data acquisition interface, battery operated compact PC (7 inch) for data acquisition, display and storage and 24V Li-ion battery (10 Ah). The two reagents and waste bottles can be either integrated within the analyzer or connected to the analyzer at the measurement site by means of small Tygon tubings. The optimization of the analyzer performance and applications will be also presented.

Keywords: Detector, Fuels\Energy\Petrochemical, Gas, Portable Instruments

Application Code: Process Analytical Chemistry

Methodology Code: Portable Instruments
Recent engineering breakthroughs in spectroscopic instrumentation resulted in worldwide acceptance of portable Raman as a valuable analytical tool. Advantages of this technique include broad applicability scope, sensitivity to chemical structure; little or no sample preparation; ability to measure directly through glass and plastic containers; as well as intrinsic suitability of Raman for field, in-line, on-line and at-line measurements.

Most of today's commercial portable Raman analyzers utilize 785- or 1064-nm laser excitation. However, the 785- and 1064-nm units still present a number of challenges, including relatively high cost per instrument as well as a relatively weak Raman signal per unit laser power that results in slow analysis. In contrast, Raman analyzers of the latest generation, which utilize other excitation wavelengths, such as 532 nm, offer a faster and less expensive alternative. The introduction of these new units was aided by recent developments in analytical methodology to minimize the impact of unwanted fluorescence on Raman measurements.

This work directly demonstrates that the 532-nm laser excitation must be revisited as a highly attractive option for portable Raman. Identified benefits of this excitation include up to 50% cost reduction per Raman unit; 5- and 16-fold faster analysis than that for 785- and 1064-nm Raman, respectively; best-in-class combination of spectral range (~120-4000 cm⁻¹) and spectral resolution (4-6 cm⁻¹); unmatched performance in water and most of organic solvents; reduced laser-induced sample degradation; superior analysis through amber, green and blue containers; and significantly reduced detection limits with improved analysis accuracy. Therefore, 532-nm portable Raman can dramatically improve business case for a number of practical laboratory, quality control, and process analytical chemistry applications, as well as extend the applicability scope of handheld Raman to new fields.
Diabetes is a metabolic disease caused by either deficiency of insulin (type 1 diabetes) or due to elevated levels of insulin (type 2 diabetes) when the pancreatic cells cannot metabolize sugar to maintain normal glucose levels. Insulin is the primary hormone that is important in maintaining continuous glucose homeostasis in the body. Global report on diabetes by the World Health Organization has found that diabetes is on a rise and its prevalence is steadily increasing in developed and developing nations over the past two decades. Diabetes of all types, if left uncontrolled, can lead to serious complications such as heart attack, kidney failure, vision loss and leg amputation.

Our objective is to develop new diagnostic assay strategies for measuring clinically relevant insulin levels in serum and whole blood by surface plasmon resonance microarrays that additionally provide binding kinetics parameters for insulin binding to surface immobilized monoclonal insulin-antibody. The developed insulin microarray sensor is highly sensitive and applicable for measuring insulin in patient samples.

Acknowledgements: This work was supported by the National Institute of Diabetes And Digestive and Kidney Diseases of the National Institutes of Health.

Keywords: Bioanalytical, Biosensors, Immunoassay
Application Code: Bioanalytical
Methodology Code: Sensors
MicroRNAs (miRs) are a class of small non-coding RNAs that regulate gene expression. Several studies are finding that the expression of specific miR combinations regulates many processes from homeostasis to disease. Currently there is a lack of analytical tools that can provide in situ analysis of cell-state specific miR combinations. Research will be presented on the fundamental biorecognition properties of a nano-assembly-based biosensor for in situ analysis of specific miR combinations. Central to the nano- assemblies function as a biosensor is a single-stranded-DNA probe that is engineered to have regions separately accommodating one blocking strand, two reporters strand, and three analyte miRs through complimentary base pairing. Two reporter strands are labelled with a pair of Förster Resonance Energy Transfer (FRET) dyes. The OFF state has one blocking strand and two reporter strands bind to the probe in such a way that the donor-acceptor dyes are outside the FRET distance. In the presence of three miRs, toe-hold initiated branch migration displaces and relocates the reporter strands to the different binding regions in the probe, such that the FRET dyes are brought within the FRET distance to turn the signal “ON”. The OFF to ON state is indicated by a change in the acceptor-to-donor ratio as the analyte miRs concentration increases. The advantage of such design is it can be engineered to accommodate as few as 3 miRs and as many as 10 miRs. We will discuss this biosensor’s detection mechanism, the design process, the thermodynamic driving forces, the selectivity, and the sensitivity.

Keywords: Biosensors, Fluorescence, Nucleic Acids, Spectroscopy
Application Code: Bioanalytical
Methodology Code: Sensors
Abstract Text
There is an immediate unmet need for a diagnostic technology that assists GPs and healthcare professionals when making point of care clinical prescribing decisions for respiratory tract infections (RTIs).

Currently there is no easy to use low-cost desktop product that is able to stratify patients presenting with the symptoms of a RTI from viral or bacterial aetiology during the timeframe of typical GP-Patient consultation. As a result, antibiotics are overprescribed and have contributed to the rise of antimicrobial resistance, which is associated with both long-term medical and economic uncertainty. Existing solutions fall outside of the limited GP-Patient consultation timeframe (PCR, microscopy), require specialist skills to operate them (PCR, microscopy and ELISA) and are expensive (PCR, microscopy).

We have developed a rapid, accurate and economical point-of-care viral diagnostic that is highly sensitive towards a bespoke common cold viral nucleotide sequence, so that individuals presenting with the symptoms of an RTI can be classified into those patients who have/ do not have the common cold. Clinical throat swabs samples are transferred to a lateral flow test strip, where aptamer-gold nanoparticle based sensing elements in combination with electronic components, such as optical sensors, easy to read displays, and custom designed embedded software interpret the results on behalf of the healthcare professional to indicate whether the patient is positive or negative for the common cold. Patients that exhibit a positive result for the presence of the rhinovirus are advised of therapy that will effectively manage their symptoms and to return if their condition exacerbate. Whereas, patients that are negative for rhinoviruses will be prescribed an appropriate antibiotic/course of therapy to treat their non-rhinoviral RTI.

We envisage our technology will pave the way forward and complement existing strategies at overcoming antimicrobial resistance.

Keywords: Bioanalytical, Biosensors, Biotechnology, Nanotechnology
Application Code: Biomedical
Methodology Code: Sensors
The highly sulfated polysaccharides dextran sulfate and pentosan polysulfate have a wide variety of biomedical applications. They are proved to be very potent and selective inhibitors of many viruses including Human Immunodeficiency Virus (HIV). Indeed, they have been identified as promising candidate drugs against HIV and have involved in clinical trials. They have also proved to have strong inhibitory effects on sexually transmitted diseases (STDs) including the most common sexually transmitted bacterial pathogen, chlamydia trachomatis. In addition, these high charge density polyanionic polysaccharides have strong inhibitory effects on infections caused by parasites including malaria. Therefore, developing simple, sensitive and fast analytical methods for the measurement of these polyions is of very high demand. Currently, there are no suitable methods available. Classical potentiometry with polyion selective electrodes have been used for the detection of these polysaccharides. However, these sensors are irreversible and limited to single use. Thus, they are not convenient for continuous monitoring purposes and are time and material consuming. We report here a reversible pulsed chronopotentiometric polyion sensor for the detection of dextran sulfate and pentosan polysulfate. It will be shown that these polyanions can be measured directly using polyanion selective electrodes as well as via titration with the natural protein, protamine. Moreover, it will be demonstrated that the binding ratios of these polyanions with protamine can be determined reliably using our pulsed chronopotentiometry with ion-exchanger-free polymer membrane polyion responsive electrodes under pulsed chronopotentiometry measuring mode.
Applying acupuncture needle to treat human disease or to maintain bodily condition has been practiced for thousands of years. Although it is widely used, the mechanisms and effects of acupuncture at the various meridians are inconclusive or equivocal in modern sciences. This study reports modification of acupuncture needle with an iron-porphyrin functionalized graphene composite for real time monitoring of nitric oxide (NO) in acupoints. The iron-porphyrin functionalized graphene was prepared, and applied to the tip surface of acupuncture needle by electrochemical deposition method. In the meanwhile, the other part of the needle was coated with insulation paste. After the nanocomposite modified needle was fabricated, highly specific and sensitive detection of NO was carried out by employing the catalytic properties of iron-porphyrin and the excellent conductivity of graphene. Real-time amperometric data showed that a limit detection of 3.2 nM in PBS solution was achieved by the developed needle. Very interestingly, the functionalized needle could be inserted into the acupoints of rats for real time monitoring of NO. It was found that the addition of L-arginine could cause a quick increase in NO in different acupoints, meaning that NO release from the acupoints was observed. We expect that this work will play an important role in exploring the mechanism of acupuncture and moxibustion treatment.
According to the World Health Organization, the production of SSFFC (substandard, spurious, falsely labeled, falsified, and counterfeit) pharmaceuticals is a vast and underreported problem. It is projected that anywhere up to 50% of the pharmaceuticals sold are low in quality in developing countries. SSFCs pose a major health threat in communities where analytical resources, such as trained pharmacists or analytical instruments, are scarce. In these areas, paper analytical devices (PADs), which involve simple colorimetric chemical tests on a paper-based device, can provide a screening method for suspect pharmaceuticals.

This research focused on the creation of PADs as an inexpensive, rapid, and easy way to screen for low quality pharmaceuticals. In a benchmark study of Nepali pharmaceuticals, PADs were used to screen hundreds of pharmaceutical samples. Samples of albendazole, amoxicillin/clavulanate, azithromycin, ciprofloxacin, and omeprazole were collected from different pharmacies in the Southern, Northern, South Eastern, South Western, and Kathmandu regions of Nepal in the summer of 2016. A UPLC chromatography method was developed to detect all five of these collected drugs as well as some potential substituted active pharmaceutical ingredients (API). Optimization of API extraction protocols was followed by development of LC-MS methods for the quantitation of APIs and identification of potential substitutions and degradation products. Agreement was shown between instrumental methods and PADs: both detected the appropriate API and the lack of substituted APIs in the Nepali samples. Quantitative results, however, proved to be more interesting.
Bacterial infection or contamination can be the reasons of death and rapid bacterial detection is required especially in clinical diagnostics and food safety. Standard bacterial detection method is culture based method and it take at least two days to deliver a result after sampling. This method also requires specific media, laboratories and trained staff. Surface-enhanced Raman scattering (SERS) is an alternative technique for the quantitative detection of bacteria using an optimal substrate. In the present study, a simple and highly selective sandwich assay was developed for fast detection of beta-hemolytic streptococcus and E.coli using swab and paper-membrane to enumerate bacteria in throat culture samples.

Herein, antibody functionalized hybrid magnetic nanoparticles were used as capture probe and 4-aminothiophenol (4-ATP) modified gold nanorod particles were used for SERS tag of bacteria. After interacting between the capture probe with pathogens having different initial cell concentrations, SERS measurements are taken. The proposed method is optimized as a fundamental for paper-based lateral flow immunoassay (LFIA) and the presence of Streptococcus and E.coli is tested by color change and SERS signal on the test line. The detection of Streptococcus by a paper-based test kit has been performed for the first time and it enables accurate and fast results. The obtained results were compared with plate counting classical methods and total detection time is less than two hour after construction of lateral flow immunoassay substrate. Our results demonstrate the potential use of this novel developed method to samples obtained by using rod swabs from different cultures to SERS and colorimetric based pathogen detection.

Keywords: Bioanalytical, Biological Samples, Clinical Chemistry, Surface Enhanced Raman Spectroscopy
Application Code: Clinical/Toxicology
Methodology Code: Sensors
Acoustophoresis Based Method for Diagnostic Applications

Acoustophoresis is a technique that aligns particles or cells into pre-determined regions within a microfluidic channel using acoustic radiation forces. Positioning of cells or particles inside microchannel depends on acoustophysical properties of cells or particles, such as density, compressibility, size, and etc. Some disease conditions such as malaria, sickle cell anemia and etc. alter the biophysical properties of cells by changing the protein network of the cytoskeleton. Concentration and separation of those infected cells from healthy population is a critical step in many clinical and diagnostic applications. Here we present our recent work on developing a microfluidic device for isolation and concentration of infected cells based on altered cell properties. We present our proof of the concept study using variety of particles and cells including polystyrene microspheres, live paramecium, Jurkat cells, and bio-functionalized silica microspheres. An acoustofluidic channel was created using photolithography and chemical etching technique and integrating with an acoustic transducer. Manipulation of model infected cells was carried out in the presence of acoustic radiative forces. Fluorescence images captured via an epi-fluorescence microscope equipped with sCMOS camera were analyzed by LabVIEW based program. Our observations suggest that cell population of our interest can be isolated by varying the strength of applied acoustic forces. Research reported here was supported by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant number P20GM103451.

Keywords: Bioanalytical, Isolation/Purification, Lab-on-a-Chip/Microfluidics, Separation Sciences
Application Code: Bioanalytical
Methodology Code: Microfluidics/Lab-on-a-Chip
The communication between cells and the environment on which cell proliferation takes place is important in cellular behavior. In order to understand the interactions among cells, substrates are needed that can closely mimic the state of cells \([\text{in vivo}]\) along with effective methods for detection of chemical messengers. Detection of many relevant biological analytes such as catecholamines typically requires a separation along with a sensitive detection mode such as electrochemical detection. In this study, we will describe the integration of microchip electrophoresis with electrochemical detection through the use of a PDMS valving chip with a polystyrene substrate base embedded with a palladium decoupler and platinum or carbon electrode. PC 12 release neurotransmitters such as dopamine and norepinephrine upon stimulation and are used as neural mimics. To improve the separation performance of our devices, this approach uses a capillary loop embedded into the base to provide a fused silica based separation. The capillary loop extends the overall application of hybrid PDMS-PS based devices for both electrochemical and fluorescence based studies. Ways of combining the analysis device with cell culture will also be discussed. A 3-dimensional scaffold for cell immobilization is more representative of the \([\text{in vivo}]\) environment of cells. We have found that the use of electrospun polymer fibers and cryogels provide a more suitable environment for cells to grow and communicate. The combination of 3-dimensional cell culture with high efficiency separations will provide a more accurate model of neural cell release and communication.

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

**Application Code:** Bioanalytical

**Methodology Code:** Microfluidics/Lab-on-a-Chip
Urinary tract infections (UTIs) are one of the most common infectious diseases caused by Escherichia coli, especially in women. The presence of clinical symptoms and results from microbiological analysis are used for diagnosis of this disease. In the developing world, the effective of healthcare unit is limited by the lack of skilled personnel and availability of sophisticated equipment, resulting in the increased morbidity. Herein, we developed a portable and inexpensive paper-based analytical device (PAD) for cultivation and biochemical test of nitrite to confirm the Escherichia coli infection on the same device. The PAD was fabricated by using a cheap filter paper and combined with a cotton sheet for supporting of bacterial growth. Using this device, the linear range for nitrite assay was in the range 0.05-1.6 mg/dL ($r^2 =0.989$). The coefficients of variation (CVs) of nitrite concentration were 9.11% and 8.53 % (n=20) when assay containing nitrite at 0.5 and 1 mg/dL, respectively. For Escherichia coli detection, this method is based on the catalytic activity between pre-immobilized substrate (5-Bromo-4-chloro-3-indolyl-$\beta$-D-glucuronide sodium salt (XG)) and $\beta$-glucuronidase from Escherichia coli to generate the blue pigment. With this device, the bacterial cells approximately $10^4$-$10^5$ CFU/mL can produce the blue color within 4 hours. Under the optimum conditions, the proposed devices are able to quantify the bacteria in the range of $10^4$-$10^7$ CFU/mL within 6 hours. In conclusion, the proposed paper device provides a rapid and cost-effective for UTIs screening and offers great promises for used in remote area.

**Keywords:** Bioanalytical, Biosensors, Lab-on-a-Chip/Microfluidics, Paper/Pulp

**Application Code:** Bioanalytical

**Methodology Code:** Microfluidics/Lab-on-a-Chip
A Reconfigurable Pipette for Customized, Cost-Effective Liquid Handling

We have developed a multichannel air displacement pipette with reconfigurable heads for non-standard liquid handling applications. Historically, multichannel pipettes have only been offered in linear configurations. These pipettes are designed for compatibility with well plates standardized for expensive, industrial liquid handling equipment. While linear multichannel pipettes enable many established assays, they do not support analytical tools with customized liquid holding geometries, specifically paper-based microfluidic devices. Using our pipette, complex paper-based microfluidic devices can be fabricated and tested without requiring i) multiple, time-consuming motions with a single-channel pipette or ii) device design limited to the configurations of traditional multichannel pipettes. With a simple and user-friendly design, this tool was created by modifying a commercial 12-channel pipette using machined and 3D-printed components. Our use of rapid prototyping equipment makes our inexpensive pipette readily adaptable for on-demand, device-specific applications. We demonstrate the quantitative capabilities of our pipette by performing calibration experiments, and the practical advantages of this tool are presented in the fabrication and use of a custom paper-based device. Our reconfigurable pipette supports the advancement of custom analytical tools with non-standard liquid handling requirements and provides an ergonomic alternative to commercial equipment for developers of these tools.

This work was supported by Tufts University. D.J.W. was supported by a DOE GAANN fellowship, and S.C.F. was supported by a NSF GRFP fellowship.

Keywords: Bioanalytical, Immunoassay, Lab-on-a-Chip/Microfluidics, Sample Handling/Automation
Application Code: Bioanalytical
Methodology Code: Microfluidics/Lab-on-a-Chip
Protein expression of specific genes varies widely from cell to cell. Consequently, it is important to understand protein expression at the single-cell level in order to gain higher resolution understanding of this variation. Such data would provide valuable information about cellular functions and pathways, as well as a better understanding of cell heterogeneity and stochasticity. In particular, single-cancer cell analysis is essential to gain a more complete understanding of tumor progression and cell heterogeneity and to provide insight about rare cells in a population that could guide treatment and therapy. However, many of the current methods for studying protein expression measure average signals from a bulk sample containing thousands to millions of cells, which may not be representative of the behavior of different subpopulations of cells in the sample. In such cases, protein expression levels of a minority cell population are masked by the majority population. We demonstrate the ability to quantify protein molecules at single-cell resolution using Single Molecule Array (Simoa) to study breast cancer cells of different subtypes. We further show that with Simoa's multiplexing capability, protein-protein interactions and changes in molecular biology at the single-cell level can be analyzed with the platform. Protein molecule profiles from single breast cancer cells will be presented along with data illuminating single-cell protein dynamics as cancer progresses.

This work was funded by DOD BC100510 (W81XWH-11-1-0814).
The large interest in nanostructures results from their numerous potential application in various areas such as biomedical sciences, food industry, agriculture, military, veterinary, and environment. Some challenging bioanalytical problems, such as sensitivity, specificity, stability, reproducibility, duration and cost of analysis and application for analyte detection in real samples can be resolved by nanostructure-based electrochemical biosensors.

The aim of this study was to evaluate the performance of electrochemical glucose biosensors based on electrodes modified with dendritic gold nanostuctures in the buffer and dilutes serum samples in order to develop sensitive and convenient amperometric glucose biosensor for biomedical application. The maximal current of the developed glucose biosensors in the presence of glucose, apparent Michaelis constant, limit of detection, linear range of glucose detection, and the influence of interfering materials were determined and compared.

Acknowledgements
This research was funded by a grant (No. SEN-15095) from the Research Council of Lithuania.

Keywords: Biosensors, Electrochemistry, Electrode Surfaces, Nanotechnology
Application Code: Bioanalytical
Methodology Code: Sensors
In recent decades, there have been hundreds of studies that provide evidence of cytokine levels changing over the course of an illness, identifying these proteins as potential biomarkers for the early diagnosis of infectious diseases such as influenza, tuberculosis, malaria, pneumonia, etc. The majority of assays for the detection of cytokines have limits of detection in the low picomolar range, which makes them undetectable until the later stages of disease progression. Influenza vaccination is an excellent model for biomarker profiling and prediction of the coordinated host innate and adaptive immune responses. By utilizing the ultrasensitive single molecule array (Simoa), we are able to measure the fluctuations of 15 human serum cytokines at ultralow concentrations upon vaccination in an IRB-approved study.
Gene detection has a great role in diagnosing several serious diseases and genetic defects, in modern clinical medicine. Analysis of gene detection platforms have a lot of limitations by using expensive machines, reagents and professional experimenters in the clinical application. A novel protein-gold nanomaterials were exploited with fast and convenient gene detection in the research. Our preliminary experiments with a heat resistant DNA binding protein (Sso7d) from Sulfolobus solfataricus. To control the orientation of Sso7d on nanoparticle surface, we will prepare a recombinant Cys-Sso7d with an extra cysteine (Cys) residue in the N-terminal by protein engineering. The data shows both Cys-Sso7d and Sso7d still remain the ability of binding DNA. Cys-Sso7d can be immobilized onto gold nanoparticles (Au NPs) with correct orientation to achieve maximum ability. The further study indicates Cys-Sso7d-Au NPs can specifically bind with the target gene fragment obtained from PCR amplification and cause varying degrees of aggregation. The specific binding will result in a change in the surface plasmon resonance absorption (~518 nm) of Au NPs. Thus, the sensing response of the developed sensor will be color change of Au NPs. Therefore, the target gene can be detected by simple visual observation or with smartphones camera function coupled with software analysis. Compared to traditional methods, our developed assay will be simple, fast, low cost, with high sensitivity and specificity. We believe this project will be able to develop very simple and user friendly sensor for gene detection as well to extend this technology to other applications such as detection of virus.
An Isothermal, Label-Free, and Rapid One-Step RNA Amplification/Detection Assay for Diagnosis of Respiratory Viral Infections

Recently, RNA viral infections caused by respiratory viruses, such as influenza, parainfluenza, respiratory syncytial virus, coronavirus, and Middle East respiratory syndrome-coronavirus (MERS-CoV), and Zika virus, are a major public health threats in the world. Although myriads of diagnostic methods based on RNA amplification have been developed in the last decades, they continue to lack speed, sensitivity, and specificity for clinical use. A rapid and accurate diagnostic method is needed for appropriate control, including isolation and treatment of the patients. Here, we report an isothermal, label-free, one-step RNA amplification and detection system, termed as iROAD, for the diagnosis of respiratory diseases. It couples a one-step isothermal RNA amplification method and a bio-optical sensor for simultaneous viral RNA amplification/detection in a label-free and real-time manner. The iROAD assay offers a one-step viral RNA amplification/detection example to rapid analysis (<20 min). The detection limit of iROAD assay was found to be 10-times more sensitive than that of real-time reverse transcription-PCR method. We confirmed the clinical utility of the iROAD assay by detecting viral RNAs obtained from 63 human respiratory samples. We envision that the iROAD assay will be useful and potentially adaptable for better diagnosis of emerging infectious diseases including respiratory diseases.
We have developed a simple solid-state synthesis procedure to functionalize fluorescent carbon quantum dots (CQDs), using ammonium citrate as a carbon source and appropriate molecules as recognition ligands. Mannose and folic acid were used to modify CQDs to selectively label Escherichia coli (E. coli) and tumor cells, respectively. First, fluorescent-core CQDs (approximate size: 3 nm) were synthesized through carbonization of ammonium citrate via dry heating. In the second step, CQDs were heated with mannose and folic acid to prepare mannose-functionalized CQDs (Man–CQDs) and folic acid-functionalized CQDs (FA–CQDs), respectively, through a dehydration reaction in the solid state. Solid-state synthesis of the self-functional CQDs is achievable without a coupling agent. We optimized the labeling efficiencies of self-functional Man–CQD and FA–CQD to cells by controlling the ratio of mannose or folic acid to CQDs, as well as the reaction temperature during synthesis. The solid-state synthesized Man–CQDs and FA–CQDs exhibited excitation–dependent fluorescence with excitation and emission maxima of 365 and 450 nm, respectively, and a fluorescence quantum yield of approximately 9%. Man–CQDs can be used for selective labeling of E. coli and detection at concentrations as low as 100 colony forming units mL⁻¹ in real samples (e.g., drinking water, apple juice, urine). Furthermore FA–CQDs are highly selective for labeling of folate receptor-overexpressing tumor cells. The synthesis of self-functional CQDs is simple, cost effective, and easily scaled up, and can be extended to the synthesis of various functional carbon nanomaterials, such as graphene oxide nanosheets, carbon nanotubes, fullerene nanoparticles and carbon nanodiamonds, with different ligands for other biolabeling applications and targeted therapies.

Keywords: Bioanalytical, Biosensors, Fluorescence, Nanotechnology
Application Code: Bioanalytical
Methodology Code: Sensors
In order to understand how individual cells respond to stimuli, single cell analysis is indispensable. Developing a device that can study response from any number of cells we want leads us to understand cellular heterogeneity deeply. The main objective of this project is to fabricate single cell arrays on porous silicon photonic crystal using poly ethylene glycol (PEG) hydrogel and photolithographic technique. By fabricating different sized pattern, different number of cells can be accommodated at precise locations. This allows us to study response from any number of cells to specific stimuli which will answer most of the unanswered questions in cell biology like why identical cancer cells behave in a different way. Why same antibiotics kill some bacteria but some still survive?

PSi is an attractive material for label-free optical biosensing due to its biocompatibility and optical sensitivity to refractive index within the pores. It has been widely used in cell based biosensing\textsuperscript{1} and in forming cell microarray\textsuperscript{2}. However, single cell sensing and study of cellular heterogeneity using this material is a novel concept.

For the biosensing purpose, matrix metalloprotease (MMPs) is chosen as it has been highlighted as a cancer biomarkers and potential therapeutics targets\textsuperscript{3}. By immobilizing particular peptide sequence as a “digestible material” within the pores, activity of specific MMP can be investigated\textsuperscript{4}. Thus the combination of selective sensor with micro-fabrication will give the ideal biosensor to map cellular heterogeneity in enzyme activity.

The authors thank CBNS (CE140100036) and ACN for support.


Keywords: Biosensors, Enzyme Assays, Fluorescence
Application Code: Bioanalytical
Methodology Code: Sensors
Specific Detection of Biomolecules in Physiological Solutions Using Polymer Modified Transistor Biosensors

Nanomaterial-based field-effect transistor (FET) sensors are capable of label-free real-time chemical detection with high sensitivity and spatial resolution, although direct measurements in high ionic strength physiological solutions remain challenging due to the Debye screening effect. Here, we present a general strategy to overcome this challenge for FET sensors, which involves incorporating biomolecule-permeable polymer layer on the device surfaces of both silicon nanowire-based and graphene-based FETs. The permeable polymer layer, polyethylene glycol (PEG), can increase the effective Debye length immediately adjacent to the device surface and thereby enable real-time detection of biomolecules in high ionic strength solutions. Concentration-dependent measurements made with these PEG modified devices exhibited real-time reversible detection of prostate specific antigen (PSA) from 1 to 1000 nM in 100 mM phosphate buffer, where the ionic strength is similar to physiological conditions. In contrast, devices without PEG modification yielded detectable signals only in low ionic strength solutions. Furthermore, co-modification of graphene FET devices with PEG and DNA aptamers showed irreversible specific binding and detection of PSA in pH 7.4 1X phosphate buffered saline solution, whereas control experiments with carcinoembryonic antigen, which does not bind specifically to the aptamer, showed much smaller reversible signals. In addition, the active aptamer receptor of the modified graphene FET devices could be regenerated to yield multi-use selective PSA sensing under these physiological conditions. We believe this work represents a critical step toward general application of nanomaterial-based FET sensors for biochemical sensing in physiological environments and thus could lead to powerful new tools for both fundamental research and healthcare applications.

CML acknowledges support from DTRA and the Air Force Office of Scientific Research.

Keywords: Bioanalytical, Biosensors, Chemical, Material Science
Application Code: Bioanalytical
Methodology Code: Sensors
Cancers are caused by mutations to genes that regulate cell normal functions. Most of the currently-available methods used for detection of DNA mismatch are time-consuming and/or require the use of fluorescent or radioactive labels. In this work, we developed a label-free enzyme reaction-based nanopore sensing approach to detect DNA mismatch. The method was rapid, accurate, and highly sensitive: picomolar concentrations of DNA could be detected in minutes. It worked on various DNA mutation situations, including base substitution, deletion, and insertion. In addition, simulated serum samples were successfully analyzed. Our developed nanopore DNA mutation detection strategy should find useful application in genetic diagnosis.
A label-free method for the detection of UO\(_2^{2+}\) ions is developed by monitoring the translocation of a peptide probe in a nanopore. Based on the difference in the number of peptide events in the absence and in the presence of uranyl ions, nanomolar concentration of UO\(_2^{2+}\) ions could be detected in minutes.

The method is highly selective; micro-molar concentrations of Ca\(_{2+}\), Cd\(_{2+}\) and Th\(_{4+}\) would not interfere with the detection of UO\(_2^{2+}\) ions. In addition, simulated water samples were also successfully analyzed.

**Keywords:** Biosensors, Environmental/Water, Metals, Peptides

**Application Code:** Bioanalytical

**Methodology Code:** Sensors
Current analytical techniques for measuring nucleic acids, and specifically RNA, rely on reverse transcription and amplification, which may lead to inaccurate quantification of the starting material. Accurate quantification of microRNAs, small non-coding RNA molecules that function in RNA silencing and regulation of gene expression, is particularly challenging due to their short length, sequence similarity, and low abundance. Our lab has developed a technique utilizing Single Molecule Arrays (Simoa) that is able to isolate and detect single molecules in femtoliter sized wells. Using Simoa, we are able to detect microRNAs directly in an ultra-sensitive and highly specific manner. We also applied our technology to measure transcript expression levels in single cells. Measuring transcript expression levels in single cells is difficult due to the low abundance of important transcripts. Copy number of transcripts can span approximately four orders of magnitude and the copy number of most transcripts is 100 copies or less per cell. Current single cell transcriptome analysis methods rely on reverse transcription and amplification by PCR. PCR amplification results in significant bias due to differential amplification efficiencies of different cDNAs, thereby confounding accurate quantification. Here, we present a novel method to detect single molecules using Simoa for ultra-sensitive direct quantification of nucleic acids.

This work was funded by DOD BC100510 (W81XWH-11-1-0814) and DARPA contract number HR0011-12-2-0001.

**Keywords:** Bioanalytical, Biosensors, Biotechnology, Nucleic Acids

**Application Code:** Bioanalytical

**Methodology Code:** Sensors
Tuberculosis (TB) is an important global health problem, particularly in developing nations. A crucial component of TB prevention and control is improving tests that can diagnose TB earlier, while maintaining good sensitivity and specificity. The gold standard tool for protein measurements, the enzyme linked immunosorbent assay (ELISA), typically has a limit of detection (LOD) in the picomolar (pM) range. Many important host response markers, such as cytokines, are not detectable since their concentrations are often below the LOD. To overcome the detection limit barrier, we employed a digital ELISA approach using Single Molecule Arrays (Simoa) to achieve highly sensitive measurements. Here, we describe a four protein host response biomarker panel developed using Simoa and applied as a blood-based diagnostic test for TB. The developed Simoa assays provided 20-500X more analytical sensitivity than the conventional ELISAs. This biomarker panel has been tested against a cohort of samples and obtained a clinical sensitivity of 89% and specificity of 71% to identify active TB from non-TB individuals, meeting the WHO guidelines. To achieve better specificity, we also developed Simoa assays for TB pathogenic protein biomarkers which are specific to M. tuberculosis (Mtbc).
Environmental Analysis of Water Quality

Evaluation of Drinking Water and Surface Water in Pennsylvania for Volatile Organic Compounds Determined by GC/MS with Purge and Trap Sample Concentration

With recent news headlines, the public has become more aware of the potential impact of water quality on human health. Two recent cases of note, Flint Michigan and Newark Public Schools, have raised public interest of what the general water quality might be in their neighborhood. While the aforementioned cases are mostly associated with metals contamination, organic compounds are also of great concern, especially in locations with higher levels of industrial activity. One such industry that has come under attention, especially in the last few years, is the shale gas drilling industry. There have been reports of potential water quality impact from drilling and hydraulic fracturing (fracking) operations, but there are no current regulatory controls regarding the use and discharge of many of the organic modifiers used in this process. Additionally, there have been reports of halogenated organics being detected that some have linked to water which has come into contact with the brine that is representative of the shale formations where the gas is being extracted. The goal of this project is to perform a high-level evaluation of volatile organics as a part of a comprehensive organics and metals water quality survey conducted in the Summer/Fall of 2016 across Pennsylvania. Specifically, this study will address the volatile organic compound (VOC) methodology utilized to perform both targeted and non-targeted analysis using purge and trap concentration coupled with gas chromatography and mass spectrometry by USEPA Method 8260. An extensive list of target compounds will be analyzed which include compounds regulated under the National Primary Drinking Water Regulations (NPDWR). Data from the PA survey study will be summarized to give an overview of the overall water quality, from a VOC perspective, which was determined in this study. Comparisons between drinking water supplies and surface waters from remote versus industrialized locations will also be presented.

Keywords: Environmental/Water, Gas Chromatography/Mass Spectrometry, Purge and Trap, Volatile Organic Compounds

Application Code: Environmental

Methodology Code: Gas Chromatography/Mass Spectrometry
Disinfection byproducts (DBP's) are a class of compounds formed through reaction of organic matter with disinfectants in the treatment process for municipal drinking water. These compounds, which include haloacetic acids (HAA's), are known carcinogens that have been regulated for many years. Drinking water in the U.S. is currently monitored for the presence of DBP's with strict regulatory limits on these compounds to ensure public safety. Of the nine HAAs commonly found (HAA9), only the most prevalent five HAAs (HAA5) are monitored. In the recently announced Unregulated Contaminant Monitoring Rule 4 (UCMR4), it was proposed that HAA6BR (brominated HAAs) and HAA9 be monitored in addition to HAA5. This presentation will describe the use of two-dimensional ion chromatography (2-D IC) to quantitate HAAs at low ppb concentrations. In the first dimension, a 4 mm i.d. column is used to separate matrix components from HAAs, which are then transferred to a concentrator column. The captured HAAs are then separated on a 0.4 mm i.d. column (capillary) with different selectivity than the first dimension column. The use of columns with different internal diameters yields enhanced sensitivity and the two different chemistries provide additional selectivity. This method requires low temperature separation (15 °C), necessitating the use of an IC system capable of maintaining sub-ambient temperature within narrow limits. With this direct injection method, recoveries for HAAs ranged from 87–116% and the lowest concentration minimum reporting levels (LCMRL) were sub-ppb, comparable to those obtained using IC-coupled to tandem mass spectrometry (EPA Method 557).
Haloacetic Acids (HAAs) can be formed during drinking water purification in municipal water supplies during the chlorination, ozonation or chloramination of water. Reactions between chlorine and organic matter present in the water can create HAAs. There are health concerns regarding human consumption of HAAs. Because of these concerns, the US EPA has published Method 557 for the quantitation of HAAs using Ion Chromatography coupled to Tandem Mass Spectrometry (IC-MS/MS). Previous techniques for the analysis of HAAs included derivitization of the HAAs and analysis by GC-MS. The IC-MS/MS method bypasses derivitization of the HAAs and allows for direct analysis of the drinking water samples without tedious and time consuming sample preparation, aside from internal standard addition and preservatives during sample collection.

Keywords: Environmental/Water, Ion Chromatography, Mass Spectrometry, Tandem Mass Spec
Application Code: Environmental
Methodology Code: Liquid Chromatography/Mass Spectrometry
Environmental Analysis of Water Quality

Determination of Toxins in Drinking Water by UHPLC/MS/MS

Microcystin congeners are a class of toxins produced by cyanobacteria, while Cyanotoxins are produced by blue-green algae, both of which can be very harmful to humans and animals especially through consumption of contaminated water. As environmental conditions change and promote harmful algal blooms growth, drinking and surface water testing becomes incredibly important to ensure a community’s safety. In this application, the water soluble and highly polar Microcystin-LR, Microcystin-RR, Cylindrospermopsin and Anatoxin-a were analyzed via UHPLC/MS/MS using a Luna Omega 1.6 \( \mu \)m Polar C18. The 100 % aqueous stability combined with enhanced polar retention provided by the Luna Omega Polar C18 allows for excellent retention of the target toxins as well as the isotopically labeled internal standards.

Abstract Text

Microcystin congeners are a class of toxins produced by cyanobacteria, while Cyanotoxins are produced by blue-green algae, both of which can be very harmful to humans and animals especially through consumption of contaminated water. As environmental conditions change and promote harmful algal blooms growth, drinking and surface water testing becomes incredibly important to ensure a community’s safety. In this application, the water soluble and highly polar Microcystin-LR, Microcystin-RR, Cylindrospermopsin and Anatoxin-a were analyzed via UHPLC/MS/MS using a Luna Omega 1.6 \( \mu \)m Polar C18. The 100 % aqueous stability combined with enhanced polar retention provided by the Luna Omega Polar C18 allows for excellent retention of the target toxins as well as the isotopically labeled internal standards.

Keywords: Environmental Analysis, Liquid Chromatography, Mass Spectrometry

Application Code: Environmental

Methodology Code: Liquid Chromatography/Mass Spectrometry
Environmental Analysis of Water Quality

**Abstract Title**  
A Sensitive Colorimetric Method for Sulphonamides Detection in Seawater Using Solid Phase Extraction and Smart phone

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Laila Idrissi, Sophia Ait Errayess

**Abstract Text**
The pharmaceutical residues received recently more attention for their presence as new emerging pollutants in fresh and marine water. Among these pharmaceuticals, sulphonamides are known to be most used antimicrobial drugs in human and veterinary medicine and thus represent a potential waste with high impact on living environment. Several methods were reported for sulphonamides detection in real samples including High Performance Liquid Chromatography (HPLC), capillary electrophoresis, Liquid Chromatography (LC-MS), Gas Chromatography, Spectrophotometry and electrochemical methods.

In this work several sulphonamide derivatives such as sulfanilamide (SAA), sulfadiazine (SDZ), sulfacetamide (SCT) sulfamethoxazole (SMX), sulfamerazine (SMR), sulfadimethoxine (SDX), sulfamethiazole (SMT) and Sulfathiazole (STZ) were determined by diazotization with sodium nitrite under acidic conditions followed by coupling agent such as N-(1-naphthyl) ethylenediaminedihydrochloride, 3-Aminophenol, diphenylamine and resorcinol to form a coloured compound.

Extraction and concentration of sulphonamides present as residues in seawater and their quantification were performed with solid phase extraction Oasis HLB cartridge (3mL, 540mg). The recovery efficiency was investigated in the sulphonamide concentration range of 0.2 to 120 ng mL-1.

Detection of sulphonamide is based on measurement of developed colour intensity either by spectrophotometer or by smart phone which is considered as new analytical tools. Almost the same reproducibility and linear range were obtained with both tools. However, smart phone is cost effective, portable and can wirelessly integrate the Internet.

The use of diphenylamine as coupling agent, Oasis HLB cartridge as extraction method and smart phone as detector makes easy determination of sulphonamide concentrations at level of ng/L in decentralized laboratories.

**Keywords:** Drugs, Environmental Analysis, Solid Phase Extraction, Spectrophotometry

**Application Code:** Environmental

**Methodology Code:** UV/VIS
Environmental Analysis of Water Quality

Photochemical Synthesis, Biological and Environmental Applications of Anisotropic Gold Nanoparticles

The demand for safer design and synthesis of gold nanoparticles (AuNPs) is on the increase with the ultimate goal of producing clean nanomaterials for biological applications. We hereby present rapid, greener, and photochemical synthesis of gold nanoparticles with sizes ranging from 10-200 nm using water soluble Quercetin Diphosphate (QDP) macromolecules. The synthesis was achieved in water without the use of surfactants, reducing agents or polymers. This approach contributes immensely to promoting the ideals sustainable nanotechnology by eradicating the use of hazardous and toxic organic solvents. The resulting triangular shaped AuNPs at 10 nm size showed significant antibacterial activity with 99.9 % inhibition towards gram (-) E.coli and gram (+) S.epidermidis compared to spherical nanoparticles. Furthermore, catalytic activities of triangular AuNPs was 3 times higher than those of the spherical AuNPs based on kinetic rate constants (k) of 3.44e-2 S⁻¹ and 1.11e-2 S⁻¹ for triangular and spherical AuNPs respectively. In this presentation, we will compare the synthetic protocols for nanoparticles using photochemical, thermal, chemical and or biogenic processes.

Keywords: Environmental/Water, Nanotechnology, UV-VIS Absorbance/Luminescence
Application Code: Environmental
Methodology Code: Process Analytical Techniques
Environmental Analysis of Water Quality

Arsenic Speciation in FGD Wastewater Samples Using Liquid Chromatography- Hydride Generation Atomic Fluorescence Spectrometry

The EPA rule for Effluent Guideline Limits (40 CFR, part 423) is an attempt to reduce the amount of toxic metals and other pollutants discharged to surface water from power plants. Previous regulations did not consider the additional burden of water discharge pollutants from air pollution control systems such as flue gas desulphurization (FGD).

The main pollutants of concern include metals (e.g Hg, As & Se), nitrogen and total dissolved solids (TDS). Toxic metal discharges have a huge impact on the environment as they bio-accumulate in wildlife and cause a wide array of human health issues.

The best available technology (BAT) that is economically achievable will need to be applied to reduce pollutant discharges. This is particularly challenging for dissolved forms of As, Se and Hg as they are not removed by surface impoundments. In addition waste streams cannot be comiled to achieve high dilution rates to meet discharge limits without first establishing pollutant levels.

Monitoring these streams is not easy because of the low concentrations that have been proposed, high dissolved solids and potential of sample matrix interference.

Online and laboratory measurements based on AFS will be used to confirm the efficiency of the wastewater treatment processes and for compliance monitoring. In this poster we will describe a system for arsenic speciation based on ion-chromatography coupled to hydride generation atomic fluorescence spectrometry. The measurement technology will be applied to the analysis of FGD wastewater samples. The importance of reliable arsenic speciation measurements in this type of sample will discussed in relation to the development of abatement systems for wastewater treatment technology. The summation of arsenic species detected will be compared to total arsenic measurements. Analytical performance data including detection limits, linearity, accuracy, precision and spike recoveries on real samples will be presented.

Keywords: Atomic Spectroscopy, Environmental/Water, Hydride, Speciation
Application Code: Environmental
Methodology Code: Atomic Spectroscopy/Elemental Analysis
**Environmental Analysis of Water Quality**

**Determination of Selenium Species in Bottled Mineral Water Causing Odour and Tainting**

Bottled water is perceived to be healthier than tap water and as such more than 75 billion gallons of bottled water is estimated to be consumed worldwide per annum. Mineral water is defined as water from underground sources and microbiologically healthy. Bottled mineral water contains characteristic bacterial flora and microorganisms that are naturally occurring. In some cases the bacteria may be introduced as a contaminant during the collecting, processing and bottling stage. No treatment can be applied to the water that changes its natural composition and as such the only processes allowed are filtration, oxygenation, settling and treatment with ozone-rich air.

The presence of volatile selenium species such as dimethylselenide and dimethyldiselenide even at part per trillion ranges may cause objectionable odours similar to rotting cabbage and garlic. The standard method of analysis based on solid phase micro-extraction with GC-MS does not offer sufficient detection limits to quantify these species at the ppt level and furthermore it does not provide determination of non-volatile selenium species. In this paper we will report our findings on the development of techniques based on Atomic Fluorescence Spectrometry for this application. Bottle water samples with and without selenium odour issues will be analysed. Samples directly from the source prior to bottling will also be tested to see whether or not the volatile selenium species are formed during storage or processing or are present at source.

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

**Application Code:** Environmental

**Methodology Code:** Atomic Spectroscopy/Elemental Analysis
The analysis of Fatty Acid Methyl Esters (FAMEs) is a critical process used in the characterization of lipid fractions in an oil. FAMEs testing allows for oil fingerprinting and authenticity analysis, and provides information that averts adulteration. Testing can present challenges that vary in complexity depending on the number of carbon atoms in the FAME, degree of unsaturation, position of the unsaturation, degree of unsaturation, geometry of the isomers.

In the present study, various separation methodologies were explored with the goal of improving resolution of similarly structured FAMEs through alteration of GC column selectivity. Simple separation of undervatized fatty acids was successful with a Zebron ZB-FFAP column phase. When derivatized, however, these compounds were more effectively separated using a polyethylene glycol phase, Zebron ZB-WAX. A new cyanopropyl-based Zebron phase with unique selectivity, ZB-FAME, was also explored for more complex FAMEs analysis. A discussion of optimized GC method development, stationary phase selectivity, and advantageous column dimensions to improve overall analysis using the new phase is presented. The experiment resulted in a short run time with complete resolution of the industry-standard 37 FAMEs mixture using a 30 meter length column.

Keywords: Capillary GC, Food Identification, Gas Chromatography
Application Code: Food Identification
Methodology Code: Gas Chromatography
Today’s demanding GC and GC/MS applications mainly focus on sensitive and reproducible qualitative and quantitative analysis of more challenging active analytes. WAX GC columns, based on 100% Polyethylene Glycol (PEG) stationary phase, are commonly used for analysis of a wide variety of compounds with polar functional groups. They are an ideal choice for a wide variety of applications in quality control and method development labs. Applications in these industries often involve complex mixtures of polar compounds containing a variety of polar functional groups.

Traditional WAX GC columns have had some shortcomings, particularly with more chemically active compounds including glycols, diols, alcohols and organic acids; they suffer from a lack of column inertness resulting in tailing peaks and variable results. Another shortcoming when using WAX GC columns is use at maximum operating temperature. In comparison to polysiloxane stationary phases the maximum operating temperature of a WAX GC column is much lower, mainly up to 250°C/260°C. More universal inertness will be shown for a variety of functional groups and range of polarities, improved thermal stability giving better column-to-column reproducibility over the life of the column.

Keywords: Analysis, Flavor/Essential Oil, Food Identification, GC Columns
Application Code: Food Identification
Methodology Code: Gas Chromatography
Microbreweries are a rapid growing industry in the United States. With this increase in microbreweries a need for quality control is also needed to ensure that the product is brewed to certain specifications. Diacetyl is a major compound that contributes to a beer’s buttery taste and aroma. Using thermal desorption and gas chromatography with mass spectrometry, the potential exists to capture volatiles at various stages in the brewing process allowing for closer analysis. The 2 step process would include sampling at the source (brewery) onto an adsorption tube and a laboratory analysis using thermal desorption and GC-MS for identification and quantitation. Previous work on the instrument assembly, design, operation, and determination of limit of detection of the diacetyl compound generated a limit of detection to be approximately 31 parts per million (ppm). Presented here is the incorporation of thermal desorption of the diacetyl and its calibration from the fermentation step of the brewing process.
Water-soluble vitamins (WSV), comprised primarily of the vitamin B complex, are essential ingredients in many foods, particularly in infant formulas. These vitamins play key factors in metabolic pathways and, therefore, impart significant health benefits when included in our daily diet.

As there are human daily nutritional recommendations for these vitamins established by the Food and Drug Administration (FDA) [sup][1][/sup], food and supplement manufacturers, as well as independent testing labs, need to be able to quantitatively verify the vitamin content in such products. When analyzing fortified foods, this can be particularly challenging due to the widely ranging concentration of vitamins. Therefore, any quantitative analytical procedure must be able to accommodate this wide spread in concentration.

With the above in mind, we present an LC-MS/MS method for the quantitative analysis of eight B-vitamins in infant formula in a single run. A simple liquid-liquid extraction was used for extracting the vitamins from infant formula. The sample extraction coupled with the fast analytical method (<6 mins) was found to be robust/reliable and the least time consuming.


**Keywords:** Food Identification, Liquid Chromatography/Mass Spectroscopy

**Application Code:** Food Identification

**Methodology Code:** Liquid Chromatography/Mass Spectrometry
A long alkyl group like C30 (triacontyl group) phase has been known to be more suitable than a conventional C18 phase for separation of hydrophobic structurally related isomers such as vitamin E or vitamin K1. However, in many case the peak using C30 column is tailing. That’s why ligand density is too high. We have reported optimization for a pore diameter of the superficially porous silica and a ligand density of C30 phase [1]. In this study, the optimized C30 phase was evaluated to compare with C18 phase or the other C30 phase. Regarding separation of cis and trans-vitamin K1, the optimized C30 phase showed better separation than the other C30 phase while a C18 phase could not separate them. The higher temperature, the worse separation of isomers. The optimized C30 phase could separate cis and trans-vitamin k at over 30 degrees Celsius although the other C30 phase couldn’t. This C30 phase was applied for separation of some structural related isomers, and showed higher resolution of isomers than conventional C18 phase.

[1] HPLC2016 P-T-0224

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Isoflavones are a small group of natural compounds that derive from 3-phenylchromen-4-one with a phenyl moiety positioned on C-3 of the chromene system instead of the usually substituted C-2 position. They are found primarily in plants of the Fabaceae family, such as soy (Glycine max), red clover (Trifolium Pratense) and Kudzu (Pueria Lobata). They are reputed to provide health benefits such as control of cholesterol and anti-cancer properties. Dietary supplements containing these compounds are widely available.

The existing industrial method (USP method) uses HPLC with UV detection to determine these compounds in dietary supplements. The run time is long (74 minutes). The peak identification relies on UV spectrum and the retention times (RT), which may not be adequate for botanic samples that may contain large number of interfering compounds with similar structure and RTs. Here we show the benefits of using UHPLC and UPLC to drastically reduce the analysis time. Also we demonstrate how the addition of a Mass Detector helps to confirm analytes' identity, avoid interference from co-eluting compounds, and improve the quantitation in accuracy and precision. Application to commercially available dietary supplements will be shown.

**Keywords:** Food Identification, Liquid Chromatography/Mass Spectroscopy, Quality Control

**Application Code:** Food Identification

**Methodology Code:** Liquid Chromatography/Mass Spectrometry
Nutritionists address benefits of obtaining elemental nutrients directly from food, especially fresh, uncontaminated produce. This project is will demonstrate non-destructive techniques developed to visually map distribution and relative abundance of elemental nutrients and treatments of produce.

Three types of X-ray fluorescence (XRF) spectrometers were used. All provided user control of atmosphere, power, filters and collimators for optimization of elemental analysis in various matrices. They also provided qualitative and quantitative XRF elemental analysis software.

1. A closed-beam benchtop 2D Micro-XRF spectrometer with 40W powered rhodium x-ray tube, silicon drift detector, programmable X-Y-Z stage, fish eye camera with two optical video microscopes, polycapillary x-ray optics for spot sizes of 25µm and software designed for collecting large elemental data sets and mapping distribution via “stitching”.

2. An open-beam portable 2D XRF spectrometer with 10W powered rhodium x-ray tube, silicon drift detector, programmable X-Y-Z stage, microscope camera and external video camera, alignment lasers, x-ray optics for spot sizes of 1mm and software designed for data acquisition and visualization of maps via “stitching”.

3. An open-beam handheld XRF spectrometer with 4W powered rhodium x-ray tube, silicon drift detector, internal VGA CMOS camera, x-ray optics for spot sizes of 3 and 8 mm and software capable of transferring acquired elemental analysis data to external mapping software to visualize maps in 2D or 3D.

Data presented will include maps of elemental nutrient rich locations in fruit as well as distribution of elemental surface treatments for preservation of produce from farm to market. The various methods utilized for data collection will be described. Additional research will include monitoring nutrient distribution changes over time and with different storage conditions.

Keywords: Elemental Analysis, Food Science, X-ray Fluorescence
Application Code: Food Science
Methodology Code: Atomic Spectroscopy/Elemental Analysis
Abstract Text
Total protein in foods and feeds is calculated using the total measured nitrogen content in the sample and a multiplier specific to the sample matrix. Nitrogen determination is commonly performed by one of two major methods—a classical wet chemistry (Kjeldahl) technique or a combustion instrument-based technique. The combustion technique is gaining popularity due to several advantages including shorter analysis times, ease of operation, and improved safety characteristics.

Total nitrogen combustion instruments use a high temperature furnace with a pure oxygen environment to combust the sample. The nitrogen gases within the sample combustion gas are subsequently reduced to N2 gas and detected using a thermo-conductivity (TC) detector, with the excess oxygen and other sample combustion products being removed using multiple reagents within the instrument flow path. Total nitrogen combustion instruments manufactured by LECO Corporation utilize a system collecting and equilibrating the combustion gas, then sampling a small aliquot of the equilibrated combustion gas for nitrogen measurement, thereby reducing the reagent demand and cost associated with treating this gas for nitrogen measurement.

This poster presentation will cover the optimization of a total nitrogen combustion instrument for lowest cost-per-analysis by optimizing the aliquot gas volume, TC carrier gas and grade of oxygen gas utilized in the combustion furnace. Data will be presented that includes common foods, feeds, and reference materials analyzed with a LECO FP628 and TruMac® N instrument.

Keywords: Agricultural, Elemental Analysis, Food Science, Protein
Application Code: Food Science
Methodology Code: Atomic Spectroscopy/Elemental Analysis
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. As a consequence, 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. Even more specialized systems as the microDSC, where bigger samples are analyzed, reach their limits due to signal saturation.

Recently, passive microrheology technique based on Diffusing Wave Spectroscopy (DWS) has been coupled with an accurate temperature control, to analyze microstructure evolution, of a complex product, under constant temperature, heating or cooling ramp. Passive microrheology studies the mobility and displacement of micron sized particles. One of the techniques used is Multi Speckle Diffusing Wave Spectroscopy (MS-DWS). The combination of this technique with an accurate temperature control provides information about the microstructure evolution during thermal processing, which can elucidate (quality) problems of food and cosmetic products. Indeed, most of these products contain fatty compounds, such as oils and waxes. These components can provoke quality problems (surface crystallization or exudation) under uncontrolled fabrication or environmental conditions.

An excellent example is the blooming of chocolate. This phenomenon occurs, when the process (or storage) conditions are not perfectly controlled. Unstable crystals are formed, which will undergo a phase transition to form big crystals on the surface, giving the chocolate a whitely aspect by reflecting the light. Unstable and stable crystals melt at different temperatures, which can be observed with thermal microrheology analyses as characteristic peaks and can therefore predict its stability against blooming.

Keywords: Cosmetic, DSC, Food Science, Thermal Analysis
Application Code: Food Science
Methodology Code: Thermal Analysis
**Abstract Text**

Thujone ([b]1[/b]) and [b]2[/b]) are present in a range of herbal products originating from plants like sage, wormwood and thyme, which produce these monoterpenes. Thujones inhibit the gamma-aminobutyric acid A (GABAA) receptor, which leads to excitations and convulsions at higher concentrations in animal studies.[sup][1][/sup] Because of its neurotoxicity maximum limits in products containing thujone have been imposed in the European Union. The metabolism of thujone is not fully understood. Investigations were performed [i]in-vitro[/i] and [i]in-vivo[/i]. On the one hand, 7-hydroxy-[b]3[/b]) was shown to be the major metabolite in in-vitro studies.[sup][1][/sup] On the other hand, [i]in-vivo[/i] studies on animals pointed to 2-hydroxy-[b]4[/b]) and 4-hydroxy-[b]5[/b]) as the main metabolites. [sup][2][/sup] To better detect trace amounts of thujone and to study the metabolism [i]in-vivo[/i] on humans, access to isotopically labelled analogues is required. Therefore, we developed a novel six step synthetic route to thujone, which enables the introduction of isotopic labels from inexpensive [i]d[/sub]6[/i]-acetone. The so formed [i]d[/sub]6[/i]-thujone ([b]1b[/b]) was also functionalized to the most important stable isotopologues of metabolites [b]3[/b]), [b]4[/b]) and [b]5[/b]). The high content in sage tea renders this beverage ideal for human studies. We present preliminary results from such studies including urine analyses.


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

**Application Code:** Food Science

**Methodology Code:** Gas Chromatography
Recently, the Food and Drug Administration announced changes to nutrition facts labels. One change includes listing the quantity of added and total sugars in food products. Changes to the sugar content label requirements is intended to help consumers of food products understand how much sugar is in different products and make health eating choices. For food producers this means that they must be able to quantify sugars in finished products and accurately list sugar content on nutritional facts label. Some sugars of interest include sugar alcohols, monosaccharides and disaccharides. Determination of individual sugars in a food product is desirable for studying nutritional value and for quality control. This work explores the use of ion exchange chromatography with pulsed amperometric detection to separate and quantify up to 11 different sugars and sugar alcohols in one run in juice and juice drinks. The use of ultrafiltration and fast analysis time of 15 minutes for all 11 sugar compounds are shown.

Keywords: Beverage, Carbohydrates, Ion Chromatography
Application Code: Food Science
Methodology Code: Liquid Chromatography
A new method based on headspace analysis performed using Vacuum Assisted Sorbent Extraction (VASE) has been developed for the determination of both volatile and semi-volatile compounds by GC/MS. Odors from thousands of volatile and semi-volatile compounds contribute to distinguishing flavors and off-odors, and the composition of these aroma compounds responsible for individual odors and flavors can be extracted and examined from the raw material to the finished product. Extraction is achieved by subjecting the sample vial to a vacuum in order to increase diffusion of aroma compounds from a complex sample matrix to the adsorbent. Compounds from a wide range of dairy products, including cheese, milk, ice cream, and yogurt have been extracted with the new vacuum assisted Sorbent Pen (SP) and analyzed using an Agilent GC/MS.

The SP evacuates the sample vial headspace directly through the adsorbent, allowing several compounds of interest to swiftly bind to the sorbent. Under vacuum, compounds transfer to the sorbent much faster due to fewer collisions between molecules. Following evacuation, a micro seal at the top of the adsorbent enables the sample vial to remain under vacuum allowing extraction to continue for a period of minutes or up to 48 hours as necessary. With far more phase capacity than SPME fibers, numerous compounds are extracted from complex matrices with minimal matrix interference. After the extraction is complete, the SP is desorbed directly onto the head of a GC column, eliminating losses.

The results show the extensive range of compounds extracted using the SP and demonstrate its potential as a routine method for examining aroma compounds relating to flavor analysis. This new technique provides a platform for several potential research projects focusing on characterizing flavor compounds of dairy products during all stages of production, packaging, and storage.

Keywords: Extraction, Flavor/Essential Oil, Food Science, Gas Chromatography/Mass Spectrometry
Application Code: Food Science
Methodology Code: Gas Chromatography/Mass Spectrometry
In light of an increasing antibiotic resistance worldwide, there is a newly awakened interest in the effect of honey on antibiotic-resistant bacteria. The antimicrobial properties of honey for the treatment of wound infections have been known since ancient times. Especially Manuka honey, derived from the Manuka tree (Leptospermum scoparium) endemic to New Zealand, is well-known for high levels of methylglyoxal (MGO), an antimicrobial active compound. In this study we analyze volatile organic compounds (VOCs) in the head-space above Manuka honey and several kinds of common honey, in order to determine MGO levels and differences in composition between the samples.

A Proton-Transfer-Reaction Time-Of-Flight Mass-Spectrometer (PTR-TOFMS) provides a comprehensive analysis of the VOC headspace profile within seconds. In this study we employ a novel type, a PTR-QiTOF, which is equipped with a Quadrupole ion guide (Qi) to transfer the ions more efficiently from the PTR ionization to the TOF mass analyzer. This increases the instrument’s sensitivity by orders of magnitude compared to a conventional setup.

We analyze MGO in the headspace of different Manuka honey samples (30, 100, 250, 400 and 550 mg MGO/kg). The figure below shows excellent correlation between the MGO content stated by the manufacturer and the measured MGO signal from the headspace analysis. In addition, we compare VOC profiles of Manuka and several other honeys. The possibility to assess Manuka honey quality by a fast headspace analysis exemplifies the potential of high sensitivity PTR-TOFMS for the rapid analysis of complex samples in food and flavor research.
Food-grade carbon dioxide has numerous applications in the food and beverage industry. It is used for carbonation of drinks, to draw beverages, as a packaging gas, for cooling, etc. In this study we investigate volatile organic impurities in a cylinder of food-grade certified (European standard E 290) CO\textsubscript{2} using Proton-Transfer-Reaction – Mass Spectrometry (PTR-MS). PTR-MS is a well-established technique for direct injection, online quantification in many fields of application (environmental research, food and flavor science, industrial monitoring, etc.). We use soft, chemical ionization by proton transfer from H\textsubscript{3}O\textsuperscript{+} coupled with a Time-Of-Flight mass spectrometer (PTR-TOF), which enables the detection and quantification of most organic compounds.

We analyzed gas from the CO\textsubscript{2} cylinder unfiltered and filtered by a charcoal filter and compared the resulting high-resolution mass-spectra. Most of the detected organic impurities had mass-to-charge ratios (m/z) above 150 Th and concentrations between 100 pptv and 10 ppbv. Analyzing the concentration vs. time profiles of the impurities (see figure) we found distinct concentration variations for different compounds. After opening of the CO\textsubscript{2} cylinder valve only one compound at m/z 69.034 quickly reaches a constant concentration, whereas other compounds reach their concentration maxima at different times. These differences can be attributed to their individual vapor pressure and the outgassing dynamics from the liquid CO\textsubscript{2} inside the cylinder and highlight the additional information provided by real-time analysis. Consequently, the time between opening the CO\textsubscript{2} cylinder and introduction of the gas into food can have an influence on the level of impurities in the final product.

Keywords: Chemical Ionization MS, Food Science, Mass Spectrometry, Time of Flight MS
Application Code: Food Science
Methodology Code: Mass Spectrometry
The Comparison of Headspace and HS-SPME Sampling Techniques to Characterize Volatiles in Wine over an Extended Period of Time

Increased production of a wine in a multitude of regions has led to increased competition as well as an upsurge in demand for quality control. Improving grape-quality and identifying overall compounds that are a direct result of grape quality give wine producers a competitive edge in flavor and aroma control. The quality of wine can be measured by flavor/aroma components making the characterization of the volatiles responsible for these constituents an indispensable task. The volatiles documented ranged from higher alcohols to molecules like fatty acids and esters. In order to efficiently analyze these volatiles, analysis was performed by GCMS via two sampling methods: headspace solid phase micro extraction (HS-SPME) and HS-SPME Arrow from CTC Analytics. Both HS-SPME and HS-SPME Arrow samples were collected over a period of time, analyzing the degradation of flavor/aroma volatiles in the headspace of wine. In this study, a comparison between the two sampling techniques and their overall ability to extract volatiles from the headspace of wine over a four month period was reported. It was determined that the HS-SPME Arrow proved to be the more effective technique. The sensitivity of the sampling method allowed for more initial volatile characterization as well as volatile identification over time. This information will give wine producers new insight to how both flavor and aroma are affected by the ever changing headspace of wine.

Keywords: Gas Chromatography/Mass Spectrometry, Headspace, SPME, Food Science
Application Code: Food Science
Methodology Code: Gas Chromatography/Mass Spectrometry
Applying High Speed Gas Chromatography for the Speciation of Fats in Foods and Edible Oils

Determination of total fat content and its speciation as saturated, unsaturated, polyunsaturated, and trans fat for nutritional labeling is primarily accomplished by derivatization of the hydrolyzed free fatty acids as methyl esters (FAMEs) followed by high resolution GC-FID analysis. In particular, AOAC method 996.06 describes sample preparation procedures and a GC-FID method for speciation of fats in a variety of food products. Due to the high complexity and very small structural difference between analytes in a typical FAME sample the preferred GC columns are 100m or longer and use a highly polar cyanopropyl stationary phase. As a result the associated GC methods are quite slow; AOAC 996.06 requires over an hour of GC run time per sample not including oven cool down.

The analysis of FAMES has become increasingly important to food science in recent years. Unsaturated and polyunsaturated fat content is perceived favorably by consumers, who as a whole, are rapidly becoming more conscious of nutritional value. At the same time, the US FDA has issued a final determination that partially hydrogenated oils (those that commonly contain trans fats) are not GRAS and thus cannot be used in food products without specific approval. Fatty acid profiles are also useful in determining origin, authenticity, and sensory attributes of edible oils by chemical fingerprinting.

This work explores using shortened narrow bore columns, high carrier gas flows, and fast oven temperature programming as routes to reduce GC run times for FAMES analysis. The trade off between separation performance and analysis time is explored with the conclusion that relatively complex mixtures of FAMES can be separated with greatly reduced analysis time.

Keywords: Food Science, Gas Chromatography, GC Columns, High Throughput Chemical Analysis

Application Code: Food Science
Methodology Code: Gas Chromatography
Human enteric viruses are the major cause of foodborne viral gastroenteritis. The disease transmission is strongly associated with virus adhesion on the surfaces of various materials involved in ready-to-eat food preparation and hand-handling. Current understanding of virus attachment/detachment to surfaces is still preliminary and qualitative. In this work, we aim to unravel the preferential virus adhesion by sensitive detection and quantification of the strength of virus adhesion on a range of food-processing materials, including glass, PVC, aluminum, HDPE and graphite. This was carried out by applying the atomic force microscope to measure and discriminate the virus adhesion forces on various surfaces using a virion attached probe. Virus surrogate MS2 was used in the study. The result was verified by plaque assay. It suggests that PVC is the most adhesive among the five materials. While the intrinsic property of a substrate plays an essential role in virus adhesion, hydrophobic substrates, e.g., PVC and graphite, are more adhesive. By spatially resolved adhesion measurements, a rough surface, such as the porous PVC and the coarsely polished aluminum foil, was found to result in a broad distribution of the adhesion forces, and the rough regions were more adhesive than the smooth regions. Thus, a hydrophilic, smooth substrate is less attractive for virus adhesion. Further studies were undertaken concerning additional environmental factors, such as surface charge and media pH, and additional materials, including artificial skin, to reveal the potential route of virus transmission.

Keywords: Atomic Force Microscopy (AFM), Bioanalytical, Food Safety, Surface Analysis
Application Code: Food Science
Methodology Code: Surface Analysis/Imaging
Teff (Eragrostis tef) is a tropical cereal well fitted to a wide range of climate and soil conditions. Beside many interesting nutritional properties it is also considered to be highly resistant to insects. Few studies have evaluated aspects of teff nutritional and toxicological parameters. The aim of this study is to analyze the amino acid composition of dried teff seeds and to check the mutagenic potential of the hydroalcoholic extract from their seed flour. Teff seeds were dried at 60 °C for 12 h and then milled until a fine powder was obtained. After acid hydrolysis, samples were filtered, neutralized and derivatized using naphthalene-2,3-dicarboxyaldehyde. Solutes were then separated by HPLC using a C-18 stationary phase and water acidified with trifluoroacetic acid (pH 2) and acetonitrile as the binary mobile phase. The column exit was coupled to a capillary of a CE equipment with LIF detection system. The following average profile of amino acids was obtained in mg.g⁻¹ of dried seeds: His, 2.5; Arg, 4.0; Ser, 4.5; Asx, 6.8; Glx, 18.9; Thr, 7.2; Gly, 3.4; Tyr, 3.5; Ala, 5.1; Met, 0.3; Val, 5.4; Phe, 5.0; Leu+Ile, 11.5 and Lys, 4.5. Mutagenic effects were evaluated using a Salmonella/microsome mutagenicity assay in TA98, TA97a, TA100, TA1535, and TA102 strains in the presence or absence of S9 mix. The hydroalcoholic extract was not able to induce mutations in any of the strains used.
Limonene content is used to classify oil of citrus rinds into different grades. The determination of limonene from industrial process streams require a method to be fast, sensitive, precise, accurate, and specific. Gas chromatographic methods for the determination of limonene are abundant throughout the literature. Both external and internal standard are used in the determination. A method with internal standard calibration is preferred due to its better precision and accuracy. The components of sweet orange (Citrus sinensis) oil include a wide variety of hydrocarbons, aldehydes, alcohols, esters, ketones, and some miscellaneous compounds. Anisole was previously used as the internal standard. However, our experimental results showed that it coeluted with impurities in sweet orange oil. Therefore, other alternative compounds including chlorobenzene, p-xylene, bromobenzene and acetophenone, which have never been detected in sweet orange oil, have been studied as potential internal standards in the current report. Our results showed that while bromobenzene coeluted with impurities in sweet orange oil, p-xylene and chlorobenzene eluted before all the components in sweet orange oil and acetophenone eluted after limonene without overlapping with impurities in sweet orange oil. Chlorobenzene and p-xylene were better choices than acetophenone because the developed methods were able to achieve required separation within two minute. Validation of the method with both chlorobenzene and p-xylene showed that the methods were linear, precise, accurate, specific and stable. A forced-degradation experiment showed that sweet orange oil was degraded by heat and the limonene content could be readily determined by the validated method.

Keywords: Analysis, Capillary GC, Food Science, Standards
Application Code: Food Science
Methodology Code: Gas Chromatography
Food Science

Enzymatic Determination of Total Polyphenol Content in Beverages Using Green Bean and Banana Crude Extracts

The consumption of antioxidants, including phenolic compounds, is considered important for preventing the oxidative damage diseases and ageing. Controlling the quality of the known phenolic compounds sources, such as beverages, is crucial for a healthy diet. The total polyphenol content (TPC) is the parameter used to estimate of plant derived products quality.

Crude plant extracts are widely known for having enzymatic activity and are applied for analysis. We used green bean crude extract as a source of peroxidase and banana crude extract as a source of phenol oxidase for enzymatic oxidation of polyphenols.

The activity of both crude plants extracts towards the polyphenol oxidation reactions has been investigated for the following substrates: catechol, gallic acid, caffeic acid, ferulic acid, and quercetin. All the substrates have been oxidized by both plant extracts, except ferulic acid which has been oxidized only by the green bean extract, which may be explained by the less specific behavior of peroxidase.

The presence of 3-methyl-2-benzothiazolinone hydrazine increased the determination sensitivity (KM values decreased by 10 times) and broadened analytical range (from 0.1-1 mM to 0.01-1 mM). Both plant extracts have been successfully applied to the TPC determination in 7 real beverages samples (tea, coffee, wine). Similarly to other enzymatic methods, found TPC content has been lower than detected by Folin-Ciocalteau (FC) method, especially for wines samples containing sulphites. The proposed method has been characterized by 5-15% RSD values, no complex probe pretreatment and fast time of analysis (1 min versus 30 min for FC method).

Keywords: Beverage, Bioanalytical, Food Science, Spectrophotometry
Application Code: Food Science
Methodology Code: UV/VIS
A new technique is presented for the analysis of volatile and semi-volatile compounds in alcoholic beverages by GC/MS. Headspace extraction is performed by placing a strong vacuum on the sample to increase the net diffusion rate from the sample to the adsorbent.

In particular, for ethanol containing beverages where the vapor pressure is low due to high affinity of organic molecules for the sample matrix, the strong vacuum helps to substantially boost recovery of volatile compounds relative to headspace extraction techniques operating at atmospheric pressure, such as with SPME and Dynamic Headspace. With Vacuum Assisted Sorbent Extraction, or VASE, the vacuum is created by pulling the gas phase out “through” the adsorbent device, thereby trapping the initial headspace compounds during the evacuation process. Vacuum can be applied just long enough to start boiling off the ethanol/water azeotrope, or evacuation can continue to eliminate most of the ethanol and water from the sample, greatly improving the phase ratios of the sorbent relative to the remaining matrix.

After evacuation, a micro seal at the top of the adsorbent leaves the sample vial and adsorbent under vacuum to allow the extraction to continue for minutes to hours, or up to 1-2 days as needed. The novel approach utilizing this self-contained vacuum extraction system also allows the vial and adsorbent to be placed in an oven to improve recovery of low volatility compounds such as Phthalates, or they can be placed in other controlled temperatures to study the aroma, fragrance, and odor profiles at various temperatures. Several alcoholic beverages will be analyzed using this technique to show sensitivity and reproducibility in the recovery of a wide range of GC compatible compounds.
Sucralose is a widely used high-potency sweetener that provides calorie reduction value to consumers in a wide variety of food products. From an analytical chemistry perspective, food products represent highly complex matrices containing many components desirable to the consumer for a food experience, but which may confound accurate chemical analyses. This study describes an overview of some successful sample preparation and analytical approaches for sucralose sweetened hot beverage products. An analytical procedure involving solid-phase extraction (SPE) and high-performance liquid chromatography coupled with charged-aerosol detector (CAD) has been developed for the determination of sucralose in a variety of food samples. Three different sample matrices (Cocoa, Coffee, and tea) were evaluated by this procedure. After extraction with water using ultrasonic mixing, extracts were cleaned up using either Waters C18 or Alumina A solid-phase extraction (SPE). The analytes were separated in gradient elution mode on C18 column and detected by CAD. To confirm the analytical method is suitable for its intended use several validation parameters, such as linearity, accuracy by standard addition, precision, and reproducibility were evaluated. A quadratic response calibration curve was established in a range 10 to 100 ppm of sucralose concentration (R^2=0.9998). The recoveries at the tested concentrations across three levels for all three matrices were in the range of 96 to 104%. The proposed method has been successfully applied to the determination of sucralose in several matrices. The procedure described here is simple, accurate and precise. Specifically, it is suitable for use when extracting and analyzing sucralose from different food matrices. For most accurate results, quantitative analysis in different food matrices should be validated on a case-by-case basis.

Keywords: Food Science, Liquid Chromatography, Sample Preparation, Solid Phase Extraction
Application Code: Food Science
Methodology Code: Separation Sciences
Introduction

Gas chromatography – mass spectrometry (GC-MS) is typically used for analysis of volatile compounds, such as aromas, flavors, fragrances and outgas. For qualitative analysis, GC-MS is a powerful method, but due to the time required for GC it is not suitable for real-time analysis in rapidly changing samples such as measuring the flavor release phenomena from spices at the moment in which they are grinded. In order to overcome this issue, we developed a closed-chamber interface device, called Volatimeship. Volatimeship combined with DART®-MS is an effective way to monitor volatile compounds as they were detected with short analysis times and high sensitivity.

Application 1: Analysis of Coffee

In this application, we measured coffee fragrance from freshly brewed coffee within seconds. We detected many ions from brewed coffee fragrance. In order to investigate a quantitative variation of each ions, we drew the extracted ion current gram. Then, many patterns of release was detected (some ions gradually increase or decrease and others are released all at once).

Application 2: Analysis of Bathing Agent

It is traditional Japanese culture to add bathing agent to one’s bath in order to aid recovery from fatigue and relieve daily stress. In this application, several bathing agents were measured. We rapidly detected many different ion kinds and were able to track the changes in their intensity.

Application 3: Analysis of Citrus Fruits

In this application, we measured the citrus fruits fragrance. Terpinene, Limonene, Linalool and Terpineol were detected as soon as the citrus fruits had been squeezed.
A method for detection and classification of five food contaminants using surface enhanced Raman spectroscopy (SERS) with novel nanosponge substrates is reported here. Newly developed SERS substrates consisting of a gold/silver alloy film deposited onto a roughened glass surface have been found to elicit viable Raman scattering from various compounds including chemicals commonly found in altered foods. Feasibility testing using a commercial Raman benchtop system with a 638 nm laser was performed on fixed volumes of pure chemical solutions deposited onto the substrate surfaces. Samples include crystal violet (antimicrobial agent), malathion (insecticide), melamine (adulterant), malachite green (antimicrobial agent), and phosmet (insecticide) in varying concentrations between 10 ppm and 0.01 ppm. Principal component analysis (PCA) was performed to assess the natural groupings among the spectral data. Successful classification rates were achieved by employing support vector machine (SVM) analysis and linear discriminant analysis (LDA) using a commercial multivariate analysis software. SVM and LDA models were validated with two discrete validation datasets (A and B); spectra collected from 10 ppm samples reported correct classification rates ranging from 92.9% to 100% for both the SVM and LDA methods. When spectra from all sample concentrations were combined a correct classification rate of 90.1% was achieved using SVM.

Keywords: Chemometrics, Food Contaminants, Food Safety, Surface Enhanced Raman Spectroscopy
Application Code: Food Contaminants
Methodology Code: Vibrational Spectroscopy
A novel base treatment followed by liquid-liquid extraction was developed to remove the derivatization reagent interference after the sample was derivatized with BSTFA (N,O-Bis(trimethylsilyl)trifluoroacetamide) for trace determination of 1-chloro-2-propanol (1C2P) and 2-chloro-1-propanol (2C1P) in a food additive by gas chromatography mass spectrometry detection (GC/MS) in selective ion monitoring mode. Due to the large volume splitless injection needed for achieving the required sensitivity, BSTFA in the derivatization sample solution interfered with the trimethylsilyl derivatives of the analytes of interest, making their quantitation not attainable. Efforts were made to decompose BSTFA while keeping the trimethylsilyl derivatives intact. Water or aqueous sulfuric acid treatment converted BSTFA into trifluoroacetamide, which partitions between aqueous and organic layers. In contrast, aqueous sodium hydroxide decomposed BSTFA into trifluoroacetic acid, which went entirely into the aqueous layer. No BSTFA or its byproduct trifluoroacetamide was found in the organic layer where the derivatized alcohols existed, which in turn completely eliminated their interference, enabling accurate and precise determination of ppb of the short-chain alcohols in the food additive. Contrary to the conventional wisdom that a trimethylsilyl derivative is susceptible to hydrolysis, the derivatized short-chain alcohols were found stable even in the presence of 1N sodium hydroxide as the improved GC/MS method was validated successfully, with a satisfactory linearity response in the concentration range of 25-400 ng/g (regression coefficient greater than 0.999), good method precision (<4%), good recovery (90-98%), and excellent limit of detection (3 ng/g) and limit of quantitation (10 ng/g).
The retention index system was proposed in 1958 by Kováts. It is based on the correlation between the retention time of the analytes and the ones of a series of references standards, making retention data dependent only on the chromatographic phenomenon, viz. on the three terms interaction analyte-stationary phase-mobile phase. Particularly in gas-chromatography (GC), because the mobile phase has a negligible influence, the retention of the analytes depends almost entirely on the stationary phase and it is as independent as possible from operating conditions. This made retention index databases usable in samples identification at both intra- and inter-laboratory level. The scope of this research is to create a similar identification system in liquid chromatography (LC), where the identification of unknown is still a challenge because of the not-availability of spectral databases related to the not reproducibility of mass spectra generated by means of atmospheric pressure ionization technique normally interfaced to LC. Lipid compounds, specifically triacylglycerols (TAGs), were selected as targeted analytes mainly due to their regular chromatographic LC profile under reversed phase conditions. The odd carbon chain number TAG series from trinonain to trinonadecanoin was chosen as the basis of the retention index scale and a database of 203 TAGs was built. For this purpose an ultra high performance LC (UHPLC) method, able to maximize the baseline separation of TAG compounds in different real-world samples (vegetable oils, fish and milk samples) was developed.
Food Science

Effects of the Iron Enrichment of Adzuki Bean ([i]Vigna Angularis[/i]) Sprouts on the Elemental Translocation and Distribution of Proteins and Fe-Metalloproteins

Iron is an essential element for human nutrition, and its deficiency or low hemoglobin levels is a global health issue. The adzuki bean ([i]Vigna angularis[/i]) is among the species of beans most recommended for the sprout cultivation and consumption, where the sprout production in rich medium is a way to supply such deficiency in nutrients. Therefore, this work aims to evaluate: (1) Fe-enriched adzuki bean sprout production; (2) iron accumulation and translocation in plants in different culture medium enriched with different masses (500, 1000, 2000, and 3000µg) of iron compounds (FeCl3, FeSO4 e Fe(III)-EDTA); and (3) effects of the enrichment in the Ca, Cu, Fe, K, Mg, S and Zn distribution in different parts of the plant (roots, stems and cotyledons), protein concentrations (albumins, globulins, prolamins and glutelins) and their association with Fe in edible plant part (stems). Cultivate of sprouts, adding deionized water (for control group) and different solutions of iron (for enriched groups) to the absorbent cotton, was done at ambient room temperature and dark conditions, for 13 days. The total elemental determination was done by inductively coupled plasma optical emission spectrometry, protein quantification by Bradford’s method, and their association with Fe by graphite furnace atomic absorption spectrometry. The enrichment with Fe(III)-EDTA favored the translocation and increased the Fe concentration in the sprouts (75 %), besides of promoting an increase in the globulins (134%), glutelins (48%) and iron interaction with albumins (141%), globulins (180%), and glutelins (93%).

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Keywords: Elemental Analysis, Food Science, Spectrophotometry
Application Code: Food Science
Methodology Code: Atomic Spectroscopy/Elemental Analysis
Beer contains hundreds of organic ingredients, with concentrations spanning many orders of magnitude. Mono- and sesquiterpenes (C10, C15 respectively) are aromatic hydrocarbons found in the essential oils of various plants, and most notably for the brewing industry, in hops. Hops provide much of the characteristic flavouring of the finished beer, so the terpene content has major impact on the final aroma and flavour. These compounds have very low odour thresholds and similar spectra using conventional 70 eV EI, making them challenging to detect and identify confidently. In this study, we examine the use of Tandem Ionisation to provide a complementary soft EI dataset, with enhancement of structurally-significant ions for improved confidence in the identification of individual terpenes.

The wide variety of matrices in the beer industry - from the original hop cones (or oils) to the final beverage - can cause a number of analytical challenges. We show how the use of thermal desorption with GC(xGC)-TOF MS can tackle this effortlessly, through a range of sample introduction techniques, including SPE, direct desorption and dynamic headspace using microchambers.
Headspace Method Comparison for GC/MS Food Analysis

Syringe and SPME Headspace analysis of food volatiles are compared using a robotic multi-purpose autosampler for ease-of-use, selectivity, method development issues, and robustness.

Volatile chemicals found in the headspace (HS) vapors of foods contribute strongly to organoleptic perception. Their absolute and relative characterization can be used for many purposes including safety assessment, formulation, quality control, competitive product analysis, source verification, and brand protection. There are several methods of headspace sampling commonly used for Gas Chromatography / Mass Spectrometry (GC/MS). This study compares automated liquid injection with headspace syringe injection and (SPME) headspace for the analysis of several foods. Ease-of-use, selectivity, method development issues, robustness and throughput are discussed.

Peppermint oil was selected as the target of comparison based on its complexity and common usage in food products. It has approximately 40 significant components covering a range of volatility and concentration. Liquid injection of peppermint oil ([i]Mentha piperita[/i]) was used to establish chromatography and identify the expected chromatographic peaks.

All three injection techniques were able to detect the complete range of peppermint oil analytes. Both headspace techniques were useful for samples of hard candy, chewing gum, tea leaves, and chocolate-covered mint cookies. Sample preparation was minimal. Differences in the chromatograms could be used to elucidate information about natural, artificial, or mixed peppermint oil composition, and localization on the exterior or interior of the food product.

Keywords: Flavor/Essential Oil, Food Science, Gas Chromatography/Mass Spectrometry, SPME
Application Code: Food Science
Methodology Code: Gas Chromatography/Mass Spectrometry
FDA requires any packaging material that contacts food or beverage products be analyzed for potential contaminants. There is also a requirement to insure that the packaging will not allow contaminants to migrate through the various packaging layers. Historically the analysis requires a solvent extraction and subsequent GC/MS validation. This work involves a novel process where the packaging material is sandwiched between two PTFE plates that are leak tight. A stream of pure nitrogen is passed through the cell onto the packaging material and desorbs off any volatile or semi-volatile components. The components are trapped on an adsorbent such as Tenax TA [acute O] that is in a thermal desorption tube attached to the exhaust port of the cell. The tube is then placed in a thermal desorption system and analyzed by GC/MS. The extraction cell allows for analysis of single side (Food or beverage side) packaging and eliminates the need for solvents. This method improves specificity and sensitivity of packaging analysis and eliminates the cost of solvent purchase and disposal. The extraction cell can also be heated. This allows for studies on the effects of temperature on packaging volatiles. Studies presented include packaging contaminants found in powdered beverages and energy bars. A contaminate profile was determined over a temperature range between 20-50C for each product in an attempt to mimic worse case storage conditions.

Keywords: Contamination, Food Science, GC-MS, Thermal Desorption

Application Code: Food Safety

Methodology Code: Gas Chromatography/Mass Spectrometry
Solid Phase Micro Extraction (SPME) is a non-exhaustive sampling technique. SPME fibers are covered with different coatings of assorted thicknesses in order to extract analytes from the sample matrix. Often the coating and thickness is dependent on the analytes of interest. The ability to automate changing SPME fibers in a sampling system is invaluable when the analytes of interest are diverse and require varied coatings for the sample extraction. The advantages of automated SPME fiber exchange will be examined in this application.
A method was developed to analyze 203 pesticides in less than 9 mins using a new benchtop gas chromatography/mass spectrometer (GC/MS). Pesticide residue analysis remains an important requirement for food and feed, and sample throughput is of high importance for laboratories looking to lower the cost of sample analysis. A mixture of 203 pesticides (GC Multiresidue Pesticide Standard #1 to #9; Restek) was analyzed on a novel benchtop GC/MS capable of 50 spectra/s with a mass range from m/z 10 to 1500. An analysis typically developed on a 30m 5MS GC column was translated to a 15m x 0.25mm i.d x 0.25 µm d.f (Restek) for high-throughput analysis. A majority of the components were resolved by chromatography and spectral deconvolution. A food commodity was spiked with a subset of 34 components (GC Multiresidue Pesticide Standard #5) relevant to the commodity to generate a calibration curve from 0.100 to 5000 ppb in matrix. All of the components were detected at the 1.0 ppb level in matrix, with the majority of components detected at 0.100 ppb, with excellent linearity to 5000 ppb. An unfortified sample was used to measure concentrations of these incurred pesticides in the food commodity.

**Keywords:** Environmental Analysis, GC-MS, Pesticides, Time of Flight MS  
**Application Code:** Environmental  
**Methodology Code:** Gas Chromatography/Mass Spectrometry
GC/MS


Analytical standards were purchased from Restek (Bellefonte, PA) including 8270 MegaMix Standard (Cat # 31850), 8270 Matrix Spike Mix (Cat # 31687), CLP 04.1 BNA Surrogate Mix (Cat # 31493), SV Internal Standard (Cat # 31206), and GC-MS Tuning Mixture (Cat # 31615). A 13 point dilution series of the 8720 MegaMix Standard was prepared from 0.01 ppm to 100 ppm, spiked with 20 ppm of the BNA Surrogate Standard, and 20 ppm of the SV Internal Standard. The GC-MS Tuning Mixture was prepared at 50 ppm, and the 8270 Matrix Spike Mix was prepared at 20 ppm. Injections were made split 20:1 to increase instrument uptime because the MS had sufficient sensitivity. The total GC run time was 16.5 mins using a ZB-SemiVolatiles 30m x 0.25mm i.d x 0.25 µm d.f (Phenomenex) for chromatographic separation. The MS mass range was from 30 to 650 m/z at 10 spectra/s. The instrument tuning and system performance evaluation showed that the system passed DFTPP tuning, peak tailing for pentachlorophenol and benzidine was less than 1.5, and 4,4'-DDT breakdown was less than 2 percent. All of the 76 components in the 8270 Megamix standard had excellent linearity over 4 orders of magnitude.

Abstract Text

EPA Method 8270 was evaluated on a new benchtop gas chromatography time-of-flight mass spectrometer (GC-ToFMS). Analytical standards were purchased from Restek (Bellefonte, PA) including 8270 MegaMix Standard (Cat # 31850), 8270 Matrix Spike Mix (Cat # 31687), CLP 04.1 BNA Surrogate Mix (Cat # 31493), SV Internal Standard (Cat # 31206), and GC-MS Tuning Mixture (Cat # 31615). A 13 point dilution series of the 8720 MegaMix Standard was prepared from 0.01 ppm to 100 ppm, spiked with 20 ppm of the BNA Surrogate Standard, and 20 ppm of the SV Internal Standard. The GC-MS Tuning Mixture was prepared at 50 ppm, and the 8270 Matrix Spike Mix was prepared at 20 ppm. Injections were made split 20:1 to increase instrument uptime because the MS had sufficient sensitivity. The total GC run time was 16.5 mins using a ZB-SemiVolatiles 30m x 0.25mm i.d x 0.25 µm d.f (Phenomenex) for chromatographic separation. The MS mass range was from 30 to 650 m/z at 10 spectra/s. The instrument tuning and system performance evaluation showed that the system passed DFTPP tuning, peak tailing for pentachlorophenol and benzidine was less than 1.5, and 4,4'-DDT breakdown was less than 2 percent. All of the 76 components in the 8270 Megamix standard had excellent linearity over 4 orders of magnitude.

Keywords: Environmental Analysis, Gas Chromatography, Time of Flight MS
Application Code: Environmental
Methodology Code: Gas Chromatography/Mass Spectrometry
GC-MS with Cold EI is based on interfacing of GC and MS with supersonic molecular beams (SMB) along with electron ionization of vibrationally cold sample compounds in SMB in a fly-through ion source (hence the name Cold EI). The Aviv Analytical 5975-SMB GC-MS with Cold EI provides enhanced molecular ions, significantly extended range of compounds amenable for analysis, much faster analysis and lower limits of detection.

However, in order to convert GC-MS with Cold EI into solutions for challenging applications we further developed and combined it with enhancement technologies including:

A. A low thermal mass fast GC for sub one minute full analysis cycle time.
B. Open Probe Fast GC-MS for real time analysis with separation and library identification.
C. Pulsed Flow Modulation GCxGC-MS with Cold EI for the combination of improved separation with best identification.
D. Provision of sample elemental formula with TAMI software that improves the quadrupole mass accuracy and combines it with isotope abundance analysis.

Selected challenging applications include:

A. A universal method for ultra-fast drug of abuse analysis was developed.
B. Isomer distribution analysis method was developed for fuel and oil characterization.
C. C4 plastic explosives origin was characterized via its plasticizers isomer distributions.
D. Universal pesticide analysis method was developed with pulsed flow modulation GCxGC-MS with Cold EI.
E. Synthetic chemical reaction products were analyzed for the optimization and determination of chemical reaction yields.
F. Triglycerides in oil and human blood were analyzed with molecular ions.

Keywords: Gas Chromatography/Mass Spectrometry
Application Code: General Interest
Methodology Code: Gas Chromatography/Mass Spectrometry
Precise characterisation of petrochemical samples is crucial for quality control, and also to understand the reactions that take place during refining processes.

Comprehensive two-dimensional gas chromatography (GCxGC) offers significant advantages over conventional chromatography for such analyses, with its vastly expanded separation space and the added benefit of highly structured groupings of compounds.

Fast acquisition speeds make time-of-flight mass spectrometry (TOF MS) the ideal partner to GCxGC. Furthermore, the elimination of mass discrimination, increased sensitivity and more compact instrument designs have made TOF MS even more amenable. However, best-practice in the petrochemical industry demands that FID is used for compound quantitation.

This study explores the use of dual detection - employing TOF MS for confident identification and FID for robust quantitation. Simple yet effective data-processing will also be deployed to show its value for enhancing productivity in any high-throughput laboratory.

Keywords: Fuels, Energy, Petrochemical, GC-MS, Petrochemical
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Gas Chromatography/Mass Spectrometry
ASTM D5769 is a widely accepted standard method used in the petroleum industry for determination of benzene, toluene, and total aromatics in finished gasolines by GC-MS. A common difficulty encountered with this method are concerns with saturation of ion source leading to nonlinearity, especially for the quantification of the high-concentration aromatic toluene. High split and low volume injections into the GC were utilized in addition to full mass range acquisition with a new benchtop time of flight mass spectrometer. Two methods of data handling were applied, with automatic peak finding and library search against commercially available NIST spectral libraries. Method requirements for sensitivity, ion ratio, and calibration linearity were satisfied using a new benchtop GC-TOFMS system, providing a solution for analysis of the aromatic compounds listed in ASTM D5769. Calibration curves were built for the standard method analytes and then applied to samples with the addition of semi-quantification for similar analytes on a sample of 93-octane gasoline, as stipulated in the method.
The hazardous constituents of cigarette smoke have attracted considerable media attention, especially with increasing regulation around the world limiting or banning smoking in public places - and even in private cars if children are present.

The recent surge in tobacco-replacement devices, such as e-cigarettes, is also driving the development of robust quality-control procedures to characterise these products. E-Cigarette solutions may contain potentially harmful chemicals, including nitrosamines and polycyclic aromatic hydrocarbons (PAHs). The presence of such chemicals naturally gives rise to some concern, and confident chemical fingerprinting is required for both research and development and regulatory purposes.

Although e-cigarettes emit less particulate matter than regular tobacco cigarettes (since no combustion takes place), they still produce a wide range of compounds at trace levels. Organic constituents of tobacco smoke have historically been analysed by gas chromatography coupled with quadrupole mass spectrometry (GC–MS). However, quadrupoles are mass filters, with a high percentage of ions being wasted, which limits sensitivity. Moreover, in selected ion monitoring (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) overcomes this issue by providing highly sensitive detection whilst acquiring full-range mass spectra, to allow both target and unknown identification in a single, rapid analysis.

This poster explores the use of a multi-functional thermal desorption (TD)-GC-TOF MS system to capture and identify whole e-cigarette emissions using a single, highly-automated platform.

Keywords: Aerosols/Particulates, Chromatography, GC-MS, Thermal Desorption
Application Code: Regulatory
Methodology Code: Gas Chromatography/Mass Spectrometry
Many industries need to store high precision assembly components or raw materials in a clean and protected environment prior to processing. Examples include semiconductor, magnetic disk storage, and scientific instrumentation. Other fields such as nanotechnology and forensics may have similar contamination concerns.

For cost, availability, convenience and minimization of cross-contamination the container of choice is often a disposable and resealable plastic bag. These bags may be rated for particulate and metal contamination, but rarely for volatile or semivolatile organics. In addition to accidental contaminants, there may also be processing-related contaminants from additives (e.g. adhesives, antioxidants, dyes, flame retardants, inks, lubricants, monomers, pigments, plasticizers, slip agents, solvents, stabilizers, and varnishes) which are often not documented by the supplier.

The food and pharmaceutical industry have numerous tests for extractables and leachables, focusing on test conditions relevant to their products. The purpose of this paper is to examine some simple tests for organic contamination of plastic bags using gas chromatography/mass spectrometry (GC/MS) for detection and identification where the food and pharma methods may not model the samples of interest or contamination modes.

Plastic bags are sampled by various methods such as off-line solvent microextraction, on-line solvent extraction, static headspace sampling, and headspace Solid Phase Microextraction (SPME) in a single automated process using a multifunctional GC autosampler. Several additives are found at sufficiently high concentrations to raise concern about possible transfer to the stored items with potentially adverse consequences.

Keywords: Contamination, Gas Chromatography/Mass Spectrometry, Polymers & Plastics, Semiconductor
Application Code: Polymers and Plastics
Methodology Code: Gas Chromatography/Mass Spectrometry
Recently, there has been an increasing shift toward use of heavy crude oils in the petroleum industry as the availability of lighter oils has decreased. Analysis by comprehensive two-dimensional gas chromatography coupled to time-of-flight mass spectrometry (GCxGC TOFMS) yields a wealth of information about the chemistries of different samples, including structural classification of hydrocarbons and identification of resins and asphaltenes. A variety of chromatographic techniques that are necessary to effectively load and separate compounds with such high boiling points are explored, with emphasis on the ability of a thermal modulator to provide the crucial second dimension of separation, which significantly increases the chromatographic space and peak capacity of the system. Specific identification of any given analyte of interest is provided by the time-of-flight mass spectrometer, utilizing the benefits of automated Peak Finding and state-of-the-art deconvolution to provide quality spectra up to m/z 1500 for comparison against well-curated commercial libraries, such as the NIST Mass Spectral Databases. The combination of multi-dimensional chromatography with full mass range data acquisition also allows for additional information on the distribution of hydrocarbon classes of paraffinic, naphthenic, aromatic, and more, for further characterization of the crude oil sample.

Keywords: Fuels\Energy\Petrochemical, Gas Chromatography/Mass Spectrometry, GC-MS, High Temperature

Application Code: Fuels, Energy and Petrochemical

Methodology Code: Gas Chromatography/Mass Spectrometry
This study describes development of a GCMS method using narrow-bore (0.15 mm ID) columns, rapid oven heating and cooling, and rapid data acquisition to decrease the runtime while maintaining the strict QC requirements of EPA methods. A sensitive fast-scanning quadrupole mass spectrometer is necessary for identification and quantitation of target compounds at sub-nanogram levels.

This study presents instrument operating conditions, and method performance statistics including linearity, accuracy, precision, and detection limits for all compounds.
Vegetation releases trace levels of biogenic volatile organic compounds (BVOCs) to deter insects and to respond to the conditions of environmental stress. The most common BVOCs include monoterpenes (C10H16) or their terpenoid derivatives (i.e. C10H16O) derived from isoprene (C5H8). Studies have shown that isoprene may react with nitrogen oxides (NOx) to produce tropospheric ozone in the presence of solar radiation. Terpenes also lead to the formation of secondary organic aerosols that contribute to particulate pollution. The large acreage of pine, cedar, oak, and other vegetation in the southeastern U.S. contributes a significant amount of terpenes that play a key role in air quality in this region and are of great interest to atmospheric scientists. This presentation is focused on the development of a method that can reliably measure the levels of both the relatively non-polar monoterpenes and the more polar terpenoid compounds like terpineol, borneol, fenchone, and linalool. The samples in a forest setting and a controlled study of cut tree branches will be collected using sorbent tubes and canisters before being analyzed by gas chromatography-mass spectrometry (GC-MS). A modified sorbent tube with derivatizing reagent is used to convert polar terpenes into more volatile substances in order to improve their analytical sensitivity. The results from both the canister and sorbent sampling methods will be compared and the method of in situ derivatization on sorbent tube will be evaluated for its suitability in characterizing the profile of terpenes in regions where tropospheric ozone and secondary organic aerosol directly impact air quality.

Keywords: Derivatization, Environmental/Air, Gas Chromatography, Mass Spectrometry
Application Code: Environmental
Methodology Code: Gas Chromatography/Mass Spectrometry
Searsia chirindensis (red currant) is a shrub in Eastern Cape of South Africa, used in folk medicine for treatment of various diseases including heart complaints, diarrhea, and rheumatism. Seasonal effects on the bioactive constituents, and bioactivities properties of the leaf essential oil (EO) and it potential for the management of infectious and oxidative – stress related diseases were studied in-vitro. The EO obtained using Clevenger apparatus was characterized by GC-MS, while its antiradical, antibacterial, and cytotoxicity properties were tested by spectrophotometric, agar diffusion and hemolytic methods respectively. Limonene (28.16%), myrcene (16.97%), caryophyllene (10.99%) were the dominant constituents found in the essential oil (EO) during autumn, while limonene (25.11%), hexamethyl octasiloxane (18.30%), pinene (15. 23 %) and piperidine (11.46%) were those prominent in summer. The EO exhibited strong antibacterial activity against some multi-drug resistant reference strains including Escherichia coli (ATCC700728), Enterococcus faecium (ATCC19434) and Staphylococcus aureus (50080). The summer’s EO was more active than autumn against test bacteria with MIC ranging between 0.25 mg/mL – 0.50 mg / mL. The IC50 for summer EO (0.40 mg/mL) showed that the antiradical strength was higher than that of autumn EO (0.60 mg/mL), BHT 0.80 mg/mL in scavenging DPPH radicals. The EO effectively reduced two other radicals (LP• and NO•) in concentration dependent-manner. The EO showed some hemolytic activity on Sheep RBC, this might be due to fragile nature of RBC, since there has never be any report on human toxicity after consumption of plant leaves or fruits in South Africa. The study suggests that apart from the indigenous uses of the extract, the EO has strong bioactive compounds, noteworthy bioactivities and may be good candidate in the search for lead compounds for the synthesis of novel potent antibiotics.
Azadirachta indica (Neem tree) of the family Meliaceae, often refer to as all purpose tree is used for a wide range of medical preparations in tropical and subtropical countries. In this study, the effects of three drying methods on the composition, antibacterial and antioxidant properties of leaf, stem and stem-bark essential oils and their potential for management of infectious and non communicable diseases were investigated in vitro. The EOs obtained by hydrodistillation from the fresh (FP), air-dried (AD), sun -dried (SD) and oven-dried (OD) plant materials at 38 0C were characterized by GC-MS, while their antioxidant and antibacterial, properties were tested by spectrophotometric and agar diffusion methods respectively. Overall the air-dried method gave the highest EO yield, and best quality EO, while the sun-dried gave the least yield in terms of bioactive terpenoids content. The prominent bioactive constituents in the essential oils were caryophyllene 30.30, thiophene 10.40 and phytol 8.40 %. The IC50 for the fresh EO (1.24 mg/mL) showed the antioxidant capacity was higher than that of AD (1.32 mg/mL), OV (2.02 mg/mL), SD (2.42 mg/mL), vitamin C (1.60 mg/mL) and comparable to α-carotene (1.26 mg/mL) in scavenging DPPH radicals. The EOs reduced remarkably three other radicals (ABTS •+, LP • and NO•) in dose dependent-manner. The EOs exhibited moderate antibacterial activity against multi-drug resistant reference bacterial strains: Escherichia coli (ATCC700728), Enterococcus faecium (ATCC19434) and Staphylococcus aureus (50080) with MIC ranging 0.550 – 0.900 mg/mL when compared to Ciprofloxacin (0.500 mg/mL) the positive control. The study suggests that apart from the traditional uses of the plant extracts, the EO has strong bioactive compounds, significate bioactivities and may be good candidate in the search for lead compounds for the synthesis of novel potent antibiotics.

Keywords: Drug Discovery, Flavor/Essential Oil, Gas Chromatography/Mass Spectrometry
Application Code: Drug Discovery
Methodology Code: Gas Chromatography/Mass Spectrometry
Hydro distillation of the leaves and floral part of Callistemon citrinus produced a clear light, pale yellow and light oils having percentage yield of 0.70 (leaf), 0.80 (flowers) and 0.50 (stem) % v/w of the wet samples. A total of twenty-six components for the leaf amounting to 96.84%, forty-one components for the flower representing 98.92% and ten components amounting to 99.98% were identified in both oil samples. The leaf oil is composed of mainly oxygenated monoterpenes (70.01%), followed by monoterpenic hydrocarbons (22.89%), sesquiterpene hydrocarbon (0.20%), and oxygenated sesquiterpenes (0.84%), the dominant constituents found in the leaf oil sample were Eucalyptol (48.98%), Pinene (20.02%), -Terpineol (8.01%), pinocarveol (5.75%), other notable components found are Pinocarvone (2.81%) and -Pinene. Minor constituents are -Fenchol (0.93%), Terpinen-4-ol (0.79%), Camphene (0.63%), -Linalool (0.57%). The oil is rich in sesquiterpene hydrocarbons (30.53%), followed by oxygenated monoterpenes (26.27%), oxygenated sesquiterpenes (24.90%) and monoterpenes hydrocarbon (15.24%), the main constituents characterizing the floral oil were Eudesmol (12.93%), Caryophyllene (11.89%), (-)-Bornyl acetate (10.02%), Eucalyptol (8.11%), Bicyclogermacrene (5.71%), Eudesmol (5.21%) and 1R- -Pinene (4.57%). Ten components were detected in the stem volatile oil; the dominant constituents were eucalyptol (56.00%), -pinene (31.03%), limonene (3.97%) and -terpineol (3.69%). The present investigation also showed that the studied plant is a potentially good source of traditional medicine and because it’s a medicinal plant, it has commercial interest in both research institutes and pharmaceutical companies for the manufacture of new drugs in the treatment of various diseases, both oils are rich in 1, 8-cineole which might be responsible for the therapeutic benefits of the plant in treating various infectious diseases.
A fully automated multi-volatile (MVM) method using sequential dynamic headspace sampling (DHS) has been developed for comprehensive analysis of aroma compounds in aqueous samples. The approach combines the complimentary attributes of both classic DHS sampling and full evaporation DHS (FEDHS) sampling to offer a much more uniform and extended enrichment of aroma compounds over the entire volatility range. Three sequential DHS samplings with replaceable traps are performed on the same sample vial followed by reverse thermal desorption of the three traps for concentration in a programmed temperature vaporizing (PTV) injector and subsequent single-run GC-MS analysis. Comparative recoveries of test aroma compounds are presented for both the single DHS samplings and the combined procedure. Applications to the determination of important aroma compounds in both fruit juices and brewed coffee will be presented.
2-Step Multi-Volatile Method (2-Step MVM) for Characterization of Aroma Compounds in Bread

Headspace gas chromatography techniques are frequently used for the analysis of aroma compounds in food due to their practical advantages of simplicity, amenability to full automation, less contamination from non-volatile constituents and elimination or reduction of solvent use. However, these techniques are more selective for volatile and/or hydrophobic compounds and result in a partial chromatogram with an under-representation of hydrophilic and/or low vapor pressure aroma compounds.

A full evaporation DHS (FEDHS) method, based on a classical full evaporation technique (FET) was demonstrated for uniform enrichment of aroma compounds in several sample types. FEDHS enables near complete vaporization and uniform recovery of aroma compounds with hydrophilic and/or low vapor pressure characteristics, while leaving most of the low volatile matrix behind. In 2014, a multi-volatile method (MVM) with sequential DHS sampling using different individual trapping conditions on the same sample was developed for uniform extraction and enrichment of a wide range of aroma compounds in aqueous samples. This 3-Step MVM approach provides a representative image of the overall volatile fraction of an aqueous sample, but one limitation is the application to fermented food samples, including alcoholic beverages, which have high concentrations of Ethanol (% levels).

In this study, we developed a 2-Step MVM approach, which enables the uniform enrichment of aroma compounds with vapor pressures (VP) <20 kPa in fermented food samples, while eliminating high concentrations of Ethanol as well as water. The performance of the 2-Step MVM was illustrated with analysis of a wide variety of aroma compounds in two different bread samples made with butter or shortening.
Aroma Office 2D (Gerstel K.K.) is an integrated software approach for simultaneous processing of both retention index (RI) and mass spectral (MS) data for rapid and improved identification of flavor compounds. The program can be integrated into Agilent Chemstation Software and searches are performed using CAS numbers of candidate compounds after library searching and corresponding automatically generated RI values. When MS signals are too weak to be used, the software allows two RI values from orthogonal columns (after GC-O organoleptic evaluation) to be cross searched in the database. This offers a very useful additional identification procedure for flavor compounds. The searchable database comprises > 10,000 compounds and offers the practicing analyst full results oriented software manipulation of RI and MS data on flavor compounds.
Introduction

Direct analysis in real time-mass spectrometry (DART®-MS) is a powerful method for analyzing low molecules. In order to expand the capability on DART-MS, we have developed a temperature-regulated thermal desorption and pyrolysis device (ionRocket). By using ionRocket gradient heating, you can analyze complex mixture materials including polymers.

Application 1: Analysis of Polyester fibers

In order to analyze condensation polymers containing ester linkages such as polyester fiber, we usually use reaction pyrolysis GC/MS analysis with a methylating agent for the improvement of decomposition efficiency and the suppression of thermal degradation products. For this application, ionRocket combined with DART®-MS analysis requires no methylating agents because DART® ionization is a soft ionization technique, as opposed to the hard ionization techniques typically used with GC/MS such as electron ionization (EI). ionRocket allows direct and rapid detection of molecular ions derived from the repeating structure of the polymer. ionRocket combined with DART®-MS simplifies the identification of different types of polyester fibers.

Application 2: Analysis of Adhesives

It is difficult to analyze the main compounds of adhesive materials after the adhesive has hardened. These compounds are consumed during the hardening reaction and the hardened materials are frequently difficult to dissolve. In this application, two-part epoxy adhesives were analyzed by ionRocket combined with DART®-MS without any sample pretreatment. We detected residual (unreacted) adhesive components within hardened epoxy resin. Moreover, ionRocket combined with DART®-MS was deemed to be suitable for optimization of hardening conditions for two-part epoxy adhesives.

Keywords: Mass Spectrometry, Polymers & Plastics, Pyrolysis, Thermal Desorption

Application Code: Polymers and Plastics

Methodology Code: Mass Spectrometry
New Opportunities for the Non-Targeted Analysis of Environmental Contaminants Using Gas Chromatography-Orbitrap Mass Spectrometry

Since the middle of the 20th century GC-MS has made a long journey towards its current status as one of the major analytical techniques used in a diverse range of applications. Despite this, GC-MS has had more than four decades to wait for a new type of mass analyzer with the potential to advance its capabilities over previously applied technology. In this presentation, almost two years after the first commercial introduction of Orbitrap GC-MS in 2015, we explore how this technology has been applied specifically to the analysis of environmental contaminants and how highly selective non-targeted acquisition can be used to enhance our approach to routine environmental analysis. Primary applications to be highlighted are the discovery of new disinfection by-products (DBPs) resulting from water treatment processes using a non targeted approach, as well as the potential for addressing the difficult analytical challenges for a complex class of emerging persistent organic pollutants: short chain chlorinated paraffins (SCCPs).

Keywords: Environmental, Gas Chromatography, Gas Chromatography/Mass Spectrometry, GC-MS
Application Code: Environmental
Methodology Code: Gas Chromatography/Mass Spectrometry
An automated dynamic headspace system based on 1 L micro-scale chambers (DHS L) was evaluated for determination of VVOC and VOC emissions from building products such as paints and plywood used indoors. VOC and SVOC sampling was performed following the ISO 16000-25 standard method. The subsequent analyses were performed in accordance with ISO 16000-9. Additionally, VVOCs such as formic acid, acetic acid, hexane, and ethyl acetate were determined. The DHS L sampler enables automated sampling from micro-scale chambers using individual Tenax TA sorbent traps followed by automated analyte determination using thermal desorption (TD)-GC/MS. Paint samples were applied to aluminum foil, placed in 1 L vessels that were continuously purged with synthetic air, and 500 mL air samples taken hourly and analyzed over a total period of 24 hours. TVOC emissions were determined as toluene equivalent; emission values for individual compounds were determined based on their respective calibration curves. For one representative sample, a 3-day emission profile was recorded. In this study, mainly aromatic compounds, aldehydes and organic acids were determined. Instrument performance was demonstrated based on an n-alkane standard mixture. The larger amount of sorbent used for collection enabled the lowering of detection limits for several compounds.

Keywords: Air, Analysis, Environmental/Air, GC-MS
Application Code: Environmental
Methodology Code: Gas Chromatography/Mass Spectrometry
Magnetic ionic liquids (MILs) are a subclass of ionic liquids that exhibit paramagnetic behavior. The paramagnetic behavior combined with characteristics of traditional ionic liquids can provide benefits in sample preparation and analysis techniques; some of these benefits include rapid isolation of the extraction phase using magnetic field and varying structures for selectivity. Several Fe(III) based MILs have been successfully used in DNA extraction and in polymerase chain reaction (PCR) analysis. In this study, six MILs are employed to examine their selectivity toward nucleic acids including double-stranded and single-stranded DNA. This new class of hydrophobic MILs is composed of the trihexyl(tetradecyl)phosphonium ([P6,6,6,14+] cation and anion which contains the hexafluoroacetylacetonate ligand chelated to Co(II), Ni(II), Mn(II), Gd(III), Dy(III), and Nd(III). DNA was extracted from aqueous solution using a dispersive droplet liquid extraction method and extraction efficiencies were subsequently determined by high performance liquid chromatography with UV detection. Further analysis is necessary to evaluate the performance of MILs in complex matrices. The reported extraction technique using MILs eliminates the need for tedious and time consuming centrifugation and washing steps, and the extraction can be accomplished within minutes unlike traditional DNA extraction methods. This study provides vital insight not only on the effectiveness of MILs but also on the role of functional groups in nucleic acid extraction.

**Keywords:** Nucleic Acids, Sample Preparation, Sampling

**Application Code:** Bioanalytical

**Methodology Code:** Sampling and Sample Preparation
The QuEChERS method has been shown to be practical for pesticide analysis on a number of different sample types and is increasingly being employed on more difficult matrices. Unfortunately some matrices either by their nature or their economic value can be difficult to analyze with just the QuEChERS method alone. In some cases, these difficult matrices or sample limited matrices show lower recoveries of the pesticides than are often observed with more traditional agricultural products. An improved QuEChERS method is proposed in which both the extraction and dispersive solid phase extraction steps are heated. Adding heat to the process increases the efficiency leading to improved pesticide recoveries. In this study a number of different heating techniques, including microwave extraction and pressurized liquid extraction combined with QuEChERS are compared. This modified QuEChERS method is found to be more effective for difficult matrices than the traditional method.

Keywords: Microwave, Pesticides, Sample Preparation
Application Code: Food Safety
Methodology Code: Sampling and Sample Preparation
Emissions from industry contribute significantly to global levels of man-made VOCs, and as a result in 2001 the European technical committee for air quality (CEN/TC 264) released standard method CEN/TS 13649, which defined a procedure for monitoring VOCs from stationary sources, such as in stack gases.

This procedure involved the collection of airborne vapours onto glass tubes packed with activated carbon, followed by elution of analytes with carbon disulfide and analysis by gas chromatography–mass spectrometry (GC–MS). However, in the years since this method was released, thermal desorption has become far more popular than solvent extraction for analysis of airborne VOCs, for reasons of analytical performance as well as practicality.

As a result of these developments, in 2014 CEN/TC 264 released a revised edition of CEN/TS 13649, which (as an alternative to solvent extraction) specifies the collection of airborne vapours onto sorbent-packed steel tubes, followed by analysis of the tubes using thermal desorption (TD)–GC–MS. Thermal desorption is now widely considered to be superior to solvent extraction for reasons of practicality and analytical performance, and its inclusion in CEN/TS 13649 brings this method into line with other national and international standard methods for VOC analysis.

This presentation will describe those advantages, and illustrate the application of the new TD method cited in CEN/TS 13649 to the analysis of VOCs in ambient air.
Phthalates are used as plasticizers in a wide range of common products and over recent years have garnered a lot of attention due to their potential negative health impacts. These impacts are so concerning that the use of some phthalates have been banned in children’s toys. The ability to quickly and accurately extract phthalates from plastics such as polyethylene is advantageous and can help aid in ensuring the safety of the products made of these materials. Presented here is a new pressurized liquid extraction method for the extraction of phthalates in polyethylene. This method has been found to be faster and more effective than other proposed methods for this application.
Polydimethylsiloxane (PDMS) is often used as a biocompatible polymer to create devices for cell culture experiments. Its hydrophobic surface, however, results in substantial non-specific adsorption of a range of hydrophobic biological molecules, particularly in relatively high surface area-to-volume PDMS-based devices. We have a specific interest in characterizing stimulation of macrophage-derived cells by lipopolysaccharide (LPS), a hydrophobic signal that promotes macrophage secretion of nitric oxide (NO), which functions as a key biogenic control point. Thus, strategies for minimizing adsorption of LPS to PDMS surfaces in miniaturized chambers are fundamental to interpreting macrophage stimulation using electrochemical detection of cellularly derived NO. To address this requirement, we have functionalized PDMS surfaces by treatment with three different silane-based groups to enhance surface hydrophilicity. Here, we present the characterization of these modifications using XPS, contact angle and other analytical methods. In addition, we performed cell studies with RAW 264.7 macrophage-derived cells using the three different fictionalized PDMS surfaces. The modifications introduced to the PDMS surface show enhancement in LPS stimulation.

Keywords: Adsorption, Bioanalytical, Polymers & Plastics, Surface Analysis

Application Code: Bioanalytical

Methodology Code: Surface Analysis/Imaging
Salting out, adding a solvent, altering the pH are various means to precipitate proteins in many bioanalytical determinations. In a subsequent two-step process, the mixture is then centrifuged and the supernatant is pipetted off to separate it from the precipitate. Also, there are instances where a longer period of incubation may be necessary before centrifugation. Sometimes there is no distinct separation layer making it difficult for technicians to draw up a clean sample. Additionally, the process is not easily automatable. The traditional process is shown in the diagram below.

A new device has been produced to eliminate the pipetting step, thereby eliminating the possible reintroduction of the precipitate into the supernatant. The device also gives the ability to automate the procedure.

The device is applicable to many other applications where transfer by pipette is necessary. This paper will show the construction of this unique device and describe its use in manual and automated procedures.

Data is presented using each of several different techniques: pH changes, solvent usage, and ammonium sulfate "salting out" to precipitate proteins. Data making comparisons of yield, time efficiency, and contamination between this newly described technique/device to the pipetting will also be presented.
Tissue samples on slides obtained from pathology labs may contain foreign particles that must be identified. Some particles can be analyzed in situ, but the presence of the proteinaceous tissue matrix, stain, and embedding media can make identification difficult. Unambiguous identification of the particles requires complete isolation from the tissue. A previous method for isolation of the foreign particles involved embedding the section in collodion to preserve the spatial orientation of the particles, moving the collodion-infused section to another substrate, dissolving the collodion, and finally digestion of the proteinaceous material with bleach. The previous method consisted of many steps, and required a high degree of proficiency at manipulating micro-samples.

This simple version is suitable for cases in which the spatial orientation of the particles is not important. A micro-drop of hexane is placed on the portion of the tissue section that contains the foreign particle(s). The hexane dissolves the embedding media, and forms a ring of media surrounding a cleared area that contains only tissue and the foreign particle(s). The tissue and foreign matter is transferred with a needle to a polycarbonate membrane filter, and the filter is floated on bleach solution; the bleach wicks up and dissolves the tissue. The filter is then placed on a water-covered fritted glass filter support, and the digested tissue and bleach are removed by vacuum, leaving a clean sample of foreign particle(s), to be prepared for further analysis. The poster will include photographs and step-by-step instructions.
The United States Environmental Protection Agency (USEPA) Method 8260 has an extensive list of analytes that can be analyzed by purge and trap sampling. Two of the more troublesome compounds on this list are Ethanol and 1, 4-Dioxane. Both of these compounds are water miscible and Selective Ion Monitoring (SIM) is required in order to detect these compounds at lower levels. The advent of SIM/Scan monitoring has made it easier to analyze for these compounds by traditional purge and trap sampling. However, due to the miscibility of the compounds and their propensity to stick to the sparge vessel of the purge and trap, purge and trap sampling needs to be optimized. This application will compare linearity, method detection limits, precision and accuracy and carryover of several purge and trap sampling parameters.

**Abstract Text**

The United States Environmental Protection Agency (USEPA) Method 8260 has an extensive list of analytes that can be analyzed by purge and trap sampling. Two of the more troublesome compounds on this list are Ethanol and 1, 4-Dioxane. Both of these compounds are water miscible and Selective Ion Monitoring (SIM) is required in order to detect these compounds at lower levels. The advent of SIM/Scan monitoring has made it easier to analyze for these compounds by traditional purge and trap sampling. However, due to the miscibility of the compounds and their propensity to stick to the sparge vessel of the purge and trap, purge and trap sampling needs to be optimized. This application will compare linearity, method detection limits, precision and accuracy and carryover of several purge and trap sampling parameters.

**Keywords:** Environmental Analysis, GC-MS, Purge and Trap, Sampling

**Application Code:** Environmental

**Methodology Code:** Sampling and Sample Preparation
The isolation of nucleic acids in the lab on a chip is crucial to achieve the maximal effectiveness of point-of-care testing for detection in clinical applications. Here, we report on the use of a simple and versatile single-channel microfluidic platform that combines homobifunctional imidoesters (HIs) for nucleic acids (both RNA and DNA) extraction using thin films, termed as the HINT system. The system is based on the adaption of HIs into non-chaotropic-based nucleic acids and the capture of reagents into a low-cost thin film platform for use as a microfluidic total analysis system, which can be utilized for sample processing in clinical diagnostics. Moreover, we assessed the use of the HINT system for the extraction of nucleic acids from various samples, including mammalian cells, bacterial cells, and viruses from tick-borne disease, and also confirmed that the quality and quantity of the nucleic acids extracted were sufficient to allow for the robust detection of biomarkers and/or pathogens in downstream analysis. Furthermore, the HINT system does not require any instruments, and has improved time efficiency, portability, and affordability. Thus, we believe that the HINT system may change the paradigm of sample processing in clinical diagnostics.
Multi-Position Electric Borate Fusion Sample Preparation and Study of Bauxite Sample Analyzed by XRF

**Introduction**

Bauxite is the world’s source of aluminum. Exploration, mine planning, processing and quality control require constant monitoring of aluminum content and concentrations of other elements Si, Fe Ti, which define the grade and value of bauxite ore.

In order to monitor the grade and value of bauxite, rapid and sturdy sample preparation followed by fast analysis must be used. This application demonstrates that fast and repeatable results can be obtained by using multi-position electric fusion followed by XRF analysis.

**Experimental Procedure**

Seven CRM’s were prepared using a Claisse TheOx® Advanced, electric fusion instrument. All samples were prepared in less than 24 minutes in a 1:10 dilution ratio using pure grade lithium borate flux without NWA.

40mm fused beads prepared were measured on PANalytical Zetium WD-XRF spectrometer with a 2.4 kW Rh-anode. Total measurement time was 7 minutes.

**Results**

Repeatability was performed by measuring one bauxite sample 20 times. The fused bead was unloaded and loaded between measurements. The STD values for repeatability confirm the stability of the XRF system. The results (table 1) show excellent agreement between certified and average measured concentrations.

The fused bead sample preparation using the TheOx Advanced was tested. Sample preparation repeatability tests using CRM, where 2 replicates were made on each of the 6 positions(table 2). Another 10 fused bead replicates were produced on the same fusion position (table 3). Considering standard deviation value includes sample preparation errors and instrumental measurement, TheOx® Advanced produces highly reproducible beads.

**Significance**

Results demonstrate that Zetium XRF in combination with TheOx® Advanced system is the perfect solution analysis of elemental composition of bauxites. With preparation time of 24 minutes and measurement time of 7 minutes major and minor compounds are analyzed with excellent accuracy and precision.

**Keywords:** Automation, Elemental Analysis, Sample Preparation, X-ray Fluorescence

**Application Code:** Process Analytical Chemistry

**Methodology Code:** Sampling and Sample Preparation
Environmental contamination related to microplastics is a growing concern across the globe. In addition to the primary concern of microplastic levels in the environment, they have also been known to absorb a variety of organic materials thus facilitating transport through wastewater systems then return to the environment. Current research shows that traditional treatment methods are not sufficient to remove this small debris from the water. The focus of this study was to develop an efficient technique to collect microplastics from the wastewater effluent, and environmental samples. The microplastics were then characterized based on size, appearance and chemical composition using Fourier Transform Infrared Spectroscopy (FTIR) and Field Emission Scanning Electron Microscopy (FESEM). These field collected samples were compared to both neat polyethylene microspheres and extracted microspheres from personal care products to observe a difference in surface characteristics and composition due to weathering effects. Both microsphere standards were then artificially weathered to imitate environmental conditions in both marine and soil conditions. These were imaged with FESEM and analyzed to observe the effect of weathering on surface area and pore size. We hypothesize that weathering will increase the pore size of microplastic spheres thus increasing the rate of sorption of organic contaminants onto the plastic.

**Keywords:** Adsorption, Environmental/Waste/Sludge, Environmental/Water, Surface Analysis

**Application Code:** Environmental

**Methodology Code:** Surface Analysis/Imaging
Urban films are film-like coatings on exterior surfaces that act as reservoirs and reaction centers for a variety of persistent organic pollutants. They are composed of both organic and inorganic components from biogenic and anthropogenic emissions. While the film composition has been studied, their morphologies and chemical profiles are strongly affected by surface and bulk reactions. These result in unique physicochemical properties that remain largely unexplored. Here, we present data that provides micro and nano-scale morphology of urban films as they develop and persist on solid surfaces. Silicon wafers are housed in passive air samplers arranged at three urban/rural locations. Substrates are exposed for timescales spanning one week to 12 months and subsequently examined using several vibrational and microscopic methods to characterize the chemical composition and morphology of urban films. This information has not been previously reported to our knowledge. Results from this study are compared to existing data for model films created in our laboratory. The native films are rough and heterogeneous, composed of sub and super-micron spheroid particles ranging from 20-90% surface coverage for the one week to 12 month samples, respectively, implying that urban films continue to develop over at least several months when environmental washing is not a factor.

Keywords: Aerosols/Particulates, Environmental/Air, Microscopy, Surface Analysis
Application Code: Environmental
Methodology Code: Surface Analysis/Imaging
Protein-ligand binding plays a key role in chemical signaling across cellular membranes(1), yet the tools available to study these processes leave many unresolved factors to fully understand the process—i.e. how chemical effects and structural changes affect the affinity of specific protein-ligand interactions.

To address these challenges, we have combined two promising biotechnologies, surface plasmon resonance (SPR) and surface enhanced Raman scattering (SERS). Due to its sensitivity and real-time performance, SPR has been widely used to analyze affinity and kinetics of molecular interactions. In addition, SERS can provide chemical specificity based on molecular structural information. Our homebuilt setup uses a sapphire prism with a gold metallic film (50nm) as a SPR platform. On the film, biotinylated thiol is deposited first, followed by flowing streptavidin-functionalized gold nanoparticles (AuNPs) solution over the Biotin. 633nm laser reflects on the prism-metal interface, then photodiode can detect the shift in the SPR angle associated with a change in the refractive index on the sample surface caused by adsorption. When streptavidin-functionalized AuNPs bind to biotin on a gold thin film, the localized electromagnetic field in the AuNPs-gold film junction can enhance Raman scattering of the molecules attached to the gold particles. The SERS signal is excited by the evanescent field arising from the SPR measurement and collected by an objective lens placed over the sample surface. Preliminary results from this system have measured the SPR sensorgram and SERS spectra of a Raman reporter molecule on streptavidin functionalized AuNPs as they bind to the biotinylated surface.

The ability to detect SPR and SERS simultaneously will be a useful tool for investigating protein-ligand interactions in the future.


Keywords: Bioanalytical, Laser, Spectroscopy, Surface Enhanced Raman Spectroscopy
Application Code: Bioanalytical
Methodology Code: Surface Analysis/Imaging
Surface Analysis/Imaging

**Advancing Tip-Enhanced Raman Spectroscopy in Ultrahigh Vacuum with Single-Molecule Resolution Scanning Tunneling Microscopy**

Tip-Enhanced Raman Spectroscopy (TERS) performed under UHV conditions affords the spatial resolution of traditional Scanning Probe Microscopy (SPM) while collecting the chemical information provided by Raman spectroscopy. By using a plasmonically active material for our scanning probe, the Raman signal at the tip-sample junction is incredibly enhanced, allowing for single-molecule probing. This enhanced signal is collected by in situ lenses to maximize numerical aperture and, thus, sensitivity. This system, further aided by the benefits of Ultra-High Vacuum, is uniquely capable of obtaining surface data that would otherwise be unobtainable with less-specialized methods. By investigating substrate structures, superstructures, and the adsorption orientations obtained from vibrational modes, we extract novel surface-chemistry data at an unprecedented spatial (<1nm) and chemical resolution.

**Keywords:** Imaging, Raman Spectroscopy, Single Molecule, Surface Analysis

**Application Code:** Nanotechnology

**Methodology Code:** Surface Analysis/Imaging
Here, luminol electrochemiluminescence imaging was firstly applied to analyze the intracellular molecule, such as glucose, at single cells. The individual cells were retained in the cell-sized microwells on gold coated ITO slide, which were treated with luminol, triton X-100 and glucose oxidase simultaneously. The broken of cellular membrane using detergent released intracellular glucose into the microwell and reacted with glucose oxidase to generate hydrogen peroxide inducing the luminol luminescence under the potential. To achieve the fast analysis, the luminescence from all the 64 individual cells on the ITO slide were imaged using CCD. More luminescence was observed on all the microwells suggested that the intracellular glucose could be detected at single cells. The starvation of cells to decrease the intracellular glucose generated less luminescence, which confirmed that our luminescence intensity was correlated with the concentration of intracellular glucose. Furthermore, this electrochemiluminescence assay was also applied to analyze the membrane cholesterol at single cells. The ECL imaging cellular membrane cholesterol either activated by low ion strength buffer or the intracellular acyl-CoA/cholesterol acyltransferase (ACAT) was calibrated by hydrogen peroxide. The experiment dates exhibited the cell heterogeneity since most of the cells analyzed had the similar active membrane cholesterol, whereas few cells had more active cholesterol. Moreover, the results demonstrated that ACAT inhibited cells had more active membrane cholesterol, while further treatment of the active cells with statins decreased the active membrane cholesterol. This electrochemiluminescence imaging platform developed will be potentially applied in analysis of more molecular in single cells to elucidate the cellular heterogeneity.
A sparse supervised learning approach for dynamic sampling (SLADS) is described for dose reduction in diffraction based protein crystal positioning. Crystal centering is typically a prerequisite for macromolecular diffraction, commonly done by X-ray diffraction mapping via raster scanning. However, this additional X-ray exposure may significantly damage the crystal prior to data collection. Dynamic sampling, in which preceding measurements inform the next location to probe for image reconstruction, significantly reduced the X-ray dose experienced by crystals during positioning. The SLADS algorithm implemented herein is designed for single pixel measurements and selects a new location to measure which maximizes the expected reduction in distortion of the image. Ground truth diffraction data were obtained for a 5 \( \mu \text{m} \) diameter beam and SLADS successfully reconstructed the image, sampling only 9% of the interior of the crystal. Using \textit{in situ} two-photon excited fluorescence microscopy measurements as a surrogate for diffraction imaging with a 1 \( \mu \text{m} \) diameter beam, the SLADS algorithm enabled image reconstruction from a 12% sampling of the interior of the crystal. When implemented into the beamline at GM/CA@APS, without ground truth images, an acceptable reconstruction was obtained with \( \sim 5\% \) of the crystal sampled. The incorporation of SLADS into X-ray diffraction acquisitions has the potential to significantly minimize the impact of X-ray exposure on the crystal by limiting the dose and area exposed during crystal positioning, using data collection hardware present in most macromolecular crystallography end stations.

Funding is gratefully acknowledged from NIH R01GM-103401-3 and R01NIGMS-106484; and from AFOSR/MURI #FA9550-12-1-0458 and AFRL/RX #FA8650-10-D-5201-0038.

**Keywords:** Detection, Method Development, Microscopy, X-ray Diffraction

**Application Code:** Bioanalytical

**Methodology Code:** X-ray Techniques
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**Abstract Text**

Humans employ metals, like zinc and copper, that are essential for enzymatic and metabolic function while other heavy metals like lead, mercury, and cadmium can be toxic and interfere with biological pathways. Some heavy metals can also have a long biological half-life which can lead to long term accumulation within the organs and significant behavioral and learning deficits. Zebrafish are a very popular model organism for investigating vertebrate development because of their known genome, translucent external fertilization, and quick embryotic development. These organisms help to gain insights in the formation and function of tissues, organ systems, and neural networks. This research focuses primarily on the development of a method to determine and eventually quantify a selected group of heavy metals in these organisms by X-ray fluorescence spectrometry in total reflection and grazing incidence modes. Analysis of histological zebrafish sections will provide information about the most likely location of the heavy metal within the fish and will aid in the identification of biochemical pathways of toxicity. Our long-term goal is to understand where the different heavy metals deposit within the zebrafish body and by selecting zebrafish we have a relatively simple and well-understood biological organism to work with.

**Keywords:** Biological Samples, Metals, Toxicology, X-ray Fluorescence

**Application Code:** Clinical/Toxicology

**Methodology Code:** X-ray Techniques
Extensive research over the past few years has been focused on the synthesis and characterization of microporous materials with high internal surface areas. Metal-Organic Frameworks (MOFs), a crystalline subset of these materials, have shown promise in a wide range of applications from chemical separations, gas storage, chemical sensing, and catalysis, to ion exchange, light harvesting, and drug delivery. This talk will include new strategies for material synthesis for separation and sequestration applications.

**Keywords:** Adsorption, Characterization, Environmental Analysis, Fuels\Energy\Petrochemical

**Application Code:** Material Science

**Methodology Code:** Separation Sciences
We created free-standing carbon molecular sieve membranes that translate the advantages of reverse osmosis for aqueous separations to the separation of organic liquids. High-performance membranes derived from carbon materials have shown excellent chemical resistance, high molecular selectivity and fast mass transport across the membrane. Carbon molecular sieve (CMS) membranes with tailored ultramicropore and micropore dimensions show both high processability of polymeric membrane and the high selectivity of inorganic membranes. CMS membranes have been proven to be effective in a variety of gas separation processes such as olefin/paraffin separation, natural gas separation and air separations. However, the low fluxes observed in CMS hollow fibers (due to porous substructure collapse during pyrolysis) hinder scale-up of CMS membranes for industrial separation applications. The performance of carbon molecular sieve separation membranes, which exploit the effect of mass transport across a selective diffusion barrier to separate molecules, can be improved by reducing the thickness of the membrane. Here, a novel post-spinning cross-linking treatment is introduced to maintain the asymmetric porous morphology in CMS hollow fiber membranes even after high temperature pyrolysis processes. A spinning process was used to prepare asymmetric poly(vinylidene fluoride) (PVDF) hollow fibers with thin selective skin layers. Cross-linking of as-spun hollow fibers “locked-in” the porous substructure and the fiber morphology remained unchanged after the cross-linking process. Moreover, this technique effectively prevented substructure collapse in asymmetric CMS hollow fiber membranes. We show that these membranes operate in “organic solvent reverse osmosis” (OSRO) separation modalities and purify p-xylene at room temperature without requiring any phase change.

Keywords: Membrane
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Separation Sciences
The high porosity and tunable surface chemistries of metal-organic frameworks (MOFs) have afforded functional properties with promising applications in catalysis and selective gas separations, capture and storage. Understanding the structure-function relationship is critical to rationally improving the performance of these systems. However, beyond the initial determination of the structure of new MOFs through single crystal X-ray diffraction, quantitative analysis of MOF structure, and how it is coupled to function or evolves during chemical/phase transformation, can be challenging.

Using synchrotron-based X-ray scattering tools, often applied in-situ under active conditions, we can gain detailed insights into the structure-function relationship in MOFs. This includes revealing unexpected and unsuspected phenomena. The presentation will describe the challenges in structure characterization of active MOF states and the new insight into the role of local defects and distortions with these systems provided by pair distribution function (PDF) and powder diffraction analysis.

Keywords: Characterization, Energy, X-ray Diffraction
Application Code: Material Science
Methodology Code: X-ray Techniques
Metal-organic frameworks (MOFs) are a class of materials with high intrinsic microporosity defined by sets of organic ligands and inorganic building blocks. In addition to the high surface area, MOFs offer unprecedented tunability, thereby enabling essential gaseous analyte selectivity based on chemical functionality, modulated by the pore functionalization and pore aperture/geometry. Furthermore, MOFs present the unique opportunity to mimic the natural photosystem for light harvesting and directional energy transfer. In the natural photosystem, high efficiency of solar energy utilization directly depends on hierarchical organization of several hundred chromophores. Such chromophore arrangement can be achieved in self-assembled MOFs. One of the great advantages of MOFs for replication of a natural photosystem is that distances and angles between chromophores can be determined by single-crystal X-ray crystallography and tuned through ligand design or variation of synthetic conditions. Using MOFs as a versatile platform, we developed a novel approach for preparation of novel systems with high efficiency of energy transfer. Upon further development, the prepared crystalline materials could foreshadow a new avenue for the engineering of new sensors, solar cells, battery anodes, or light-emitting diodes in the future.

Keywords: Fluorescence, Luminescence, Spectroscopy, X-ray Diffraction
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Fluorescence/Luminescence
State-of-the-art research in the chemical sciences is often limited by the capabilities of and access to instrumentation able to perform key measurements. In September, 2016 a workshop was held to evaluate the needs and opportunities for chemical instrumentation with aggregate cost between $4 Million and $120 Million to advance research in the chemical sciences. Our efforts focused specifically on needs and opportunities associated with the creation of regional instrumentation centers that would provide individual instruments, suites of instruments, and/or cyber-enabled tools to address grand challenges in chemistry. In this talk I will discuss the overall consensus of the chemistry community in this area, as well as identified targets of opportunity.
State-of-the-art research in the chemical sciences is often limited by the capabilities of and access to instrumentation able to perform key measurements. In September, 2016 a workshop was held to evaluate the needs and opportunities for chemical instrumentation with aggregate cost between $4 Million and $120 Million to advance research in the chemical sciences. Our efforts focused specifically on needs and opportunities associated with the creation of regional instrumentation centers that would provide individual instruments, suites of instruments, and/or cyber-enabled tools to address grand challenges in chemistry. In this talk I will discuss the broader impacts this opportunity could present to the chemistry community in terms of impact on cross-disciplinary research as well as education and training of future scientists.
The limits of knowledge in chemistry are often controlled by the limits of the instrumentation used to probe the composition, structure, and dynamics of chemical systems. This talk will report the results from a 2-day workshop to address the need for a mid-scale instrument development program in the chemical sciences to support activities in the design, development, testing, and characterization of chemical instrumentation of a scale and complexity that cannot be addressed through existing programs of instrument support by federal agencies. Currently, chemical instrumentation is one focal area for the Chemistry Division of the National Science Foundation (NSF) among others, but work on instrument development is supported largely through individual grants and/or existing mechanisms, such as the Major Research Instrumentation program. The goal of the proposed Workshop on Chemical Sciences Needs for Mid-Scale Instrument Development will be to explore unmet needs and challenges related to mid-scale instrument development, requiring resources beyond those available from individual grants and other existing instrumentation programs. The relationship of the recommendations of this workshop to the Chemical Instrument Center workshop will also be
Accurate metabolite identification is a persistent problem in metabolomics, and new approaches are needed to reduce this bottleneck. Often, the identification task is improved by the combined use of NMR and MS data. However, the ability to compare and connect metabolite data between NMR and mass spectrometry (MS) can be challenging. To help alleviate this problem, we are developing a variety of “smart isotope tags” that enable improved identification of metabolites by improving the ability to connect these two analytical platforms. Statistical methods such as ratio analysis of NMR spectroscopy (RANSY) and hetero-spectroscopic approaches (RANHSY) help in the process of identification. In addition, a new approach we call “eRANSY” uses solvent extraction to simplify the NMR spectra and yields better ratio analysis results for improved unknown identification.

Another challenging problem in metabolomics is accurate quantitation, which is a major challenge for MS owing to numerous factors related to the need for isotope labeled internal standards. A new method, in which metabolites from a single serum specimen are quantitated using NMR, is being developed. MS concentrations thus derived for human serum samples exhibited excellent correlation with NMR (R² > 0.99) with a median CV of 3.2%. The NMR guided MS quantitation approach is simple, easy to implement and, by obviating the need to internally standardize each metabolite it offers a new avenue for quantitation of blood metabolites using MS, routinely. Intriguingly, some metabolites do not correlate well for different and specific reasons, which we are starting to understand. Such cases point to the need to be careful about the assumption that metabolites are generally stable during MS analysis, while opening a new avenue for identification of metabolites that are potentially fragile to ionization sources during MS analysis. This knowledge can be critical for biomarker discovery and biological interpretation.

Keywords: Bioanalytical, Liquid Chromatography/Mass Spectroscopy, Metabolomics, Metabonomics
Application Code: Bioanalytical
Methodology Code: Mass Spectrometry
The reverse engineering of genetic, proteomic, and metabolomic networks from biological data is one of the most important and challenging “knowledge extraction” tasks in bioinformatics. Gaussian graphical models (GGMs) have been proven to be a very powerful formalism to infer biological networks. Standard GGM selection techniques can unfortunately not be used in the “small N, large P” data setting. Various methods to overcome this issue have been developed based on regularized estimation, partial least squares method, and limited-order partial correlation graphs. Several studies compared the performances among several network construction algorithms, such as PLSR, SCE, and ES, ICR and PCR, Ridge regression, Lasso and adaptive Lasso, to see which method is the best for biological network constructions. Each comparison analysis resulted in that each construction method has its own advantages as well as disadvantages according to different circumstances, such as the network complexity. However, it is almost impossible to recognize the complexity of the network before estimation. Thus, we develop an Ensemble method which is model averaging to construct a metabolic network.

**Keywords:** Bioinformatics, Mass Spectrometry, Metabolomics, Metabonomics, Statistical Data Analysis

**Application Code:** Biomedical

**Methodology Code:** Data Analysis and Manipulation
Despite having free access to multiple metabolomics databases (HMDB, Metlin, KEGG, PubChem), most non-targeted discovery metabolomics studies report the identification of <35% of all detectable metabolites. HMDB and Metlin are state-of-the-art metabolomics databases that include nearly all known human biochemical compounds. It is therefore alarming that our understanding of the small molecule composition of typical human biofluids (e.g. urine or serum) is so profoundly incomplete. This leads to a current paradox within the field of metabolomics: we cannot identify the structures of unknown metabolites if they are not present in databases and we cannot add new metabolites to these databases without first identifying them. Although metabolomics researches have access to the largest freely available chemical database in the world (PubChem, with >50x10^6 chemical compounds), there is currently no way to effectively search this resource. In order for metabolomics research to make a sustained and lasting contribution to human health we need better methods for efficiently searching large biochemical/chemical databases and, biochemical databases that contain [nearly] all possible biochemical compounds (known and unknown). In this talk I will provide a brief overview of: 1) current methods of using databases for identifying the structures of unknown metabolites; 2) current methods of augmenting metabolomics databases with unknown biochemical compounds; and, 3) approaches for identifying compounds even if they are not in databases.
We have developed two software packages MetSign and IsoGC to respectively analyze the SIAM data acquired on LC-MS and GC-MS. The experimental data acquired on each analytical platform are respectively analyzed for spectrum deconvolution, isotopologue assignment, peak list alignment, normalization and statistic significance tests. The analysis results are then integrated to investigate for metabolite association network and pathway analysis. A suite of data analysis algorithms are developed to assess the quality of experiment data acquired on different analytical platforms. The developed bioinformatics platform has been validated by analysis of experimental data of mixtures of metabolite standards as well as biological samples.

Keywords: Bioanalytical, Bioinformatics, Metabolomics, Metabonomics
Application Code: Bioanalytical
Methodology Code: Data Analysis and Manipulation
Bioinformatics: Metabolite Identification and Quantification

Bioinformatics Tool Box for Mass Spectrometry Imaging of Metabolites

Mass spectrometry imaging has become one of the rapidly growing technological science. This is a non-targeted chemical imaging technique with the capability of revealing unprecedented details of biological systems. It has been applied for various biological systems, from mouse brain tumors to bacterial colonies and plant tissues, visualizing proteins, lipids, and metabolites. There are several obstacles that interfere with its true potential, especially identifying and quantifying metabolites directly on tissues.

Our group has been working on single cell level high-spatial resolution mass spectrometry imaging of plant metabolites in the past eight years. We have improved the spatial resolution from fifty to five micron by modifying the laser optics of a commercial MALDI-MS and applied for various plant tissues, including Arabidopsis flower, cotton seed, germinating maize seed, developing maize leaf, and infected rice and soybean leaves. In 'multiplex MS imaging' technique we developed, we can acquire MS and MS/MS spectra in a single MS imaging experiment. Metabolite identification can be performed by chemical composition analysis in high-resolution MS and structural analysis in MS/MS. Incorporating several matrices for consecutive tissues sections, we have demonstrated MS imaging can be applied for the metabolomics scale (Figure).

Bioinformatics in MS imaging has been exploded in the last few years: a common MS imaging data format is established, imzML, and adopted to almost all instrument types; many visualization tools are now available including several open source codes; principle component analysis and hierarchical analysis have been adopted for differential localization of chemical compounds; and quantitative visualization and fusion with optical imaging have become possible. In this presentation, we will share our experience as experimentalists with general audience in bioinformatics field, and discuss the current state of art and future needs in bioinformatics tool box for MS imaging.

Keywords: Bioinformatics, Imaging, Mass Spectrometry, Metabolomics

Application Code: Bioanalytical
Methodology Code: Mass Spectrometry
In 2009 the National Academy of Sciences criticized several forensic investigation methods as more craft than science and has asked for development of scientific techniques for identification of crime scene evidences. To address this need, the research in my lab is focused on the development and application of information rich technologies to investigate a broad array of challenges in forensic sciences. These technologies will provide alternatives to the less reliable presumptive techniques that rely on data such as color change, pattern matching, or retention time alone that are prone to more false positives. Our research plan is to package these technologies into portable devices for on-site, real-time identification of forensic evidence with information rich technologies. Analysis of forensic evidence by information rich technologies such as mass spectrometry (MS). Mass spectrometry is one of the fastest growing areas in forensic analysis. To provide more accurate identification of forensic evidence, in the past few years there has been a growing interest in moving this technology to the field for on-site, real time analysis. Several portable mass spectrometers and Raman instruments have been introduced for onsite analysis. Amongst commercially available portable mass spectrometers, several are capable of operation with atmospheric pressure ionization sources such as electrospray ionization. However, to date very few portable separation devices are available and those than could also separate the optical isomers are rare. In early 2016, we introduced a battery operated capillary electrophoresis electrospray ionization (CE/ESI) source for mass spectrometry with optical isomer separation capability. This new source provides high resolution separation, including optical isomer resolution in ~1 min. In this presentation, the application of this technique to a wide range of illicit drugs, as well as their structural and optical isomers will be presented.
The synthetic and analytical properties of a series of substituted indoles related to the cannabinoid drug of abuse 3-(1-naphthoyl)-1-pentylindole (JWH-018) will be compared in this report. Our research has recently evaluated the 1-pentylindoles having the acyl group substituted at all six of the remaining available indole ring substitution positions. Synthetic and analytical comparisons will be made for the six benzoyl, the six (1-naphthoyl) and the six (2-naphthoyl)-1-pentylindole regioisomers. These studies will focus on synthesis, chromatographic, mass spectral and infrared evaluation of these compounds.

The EI-MS for these substances show a number of equivalent regioisomeric major fragments resulting from cleavage of the groups attached to the central indole nucleus. The characteristic (M-17)+ fragment ion resulting from the loss of the OH group is significant in the mass spectra of several of the (1-naphthoyl)-1-pentylindoles but not present in significant abundance in the 2-naphthoyl or benzoyl analogues. Synthesis and analytical studies on the three regionally deuterated analogues of JWH-018, 3-(1-naphthoyl)-1-(D11)pentylindole, 3-(1-(D7)naphthoyl)-1-pentylindole and 3-(1-naphthoyl)-1-pentyl(D5)indole allow confirmation of the structure for many of the major mass spectral fragment ions in these indole derivatives including the (M-17)+ fragment ion.

Recently, we have evaluated the 3-(1-naphthoyl)-1-pentylindoles having additional groups substituted at the remaining five available indole ring substitution positions. These studies include groups such as methyl, methoxy and chloro substituted at positions 2-, 4-, 5-, 6-, and 7-of the indole ring. Most of these regioisomeric series show excellent GC separation on common stationary phases.

The overall goal of these studies is to provide an analytical framework for the identification of individual substituted indoles to the exclusion of all other possible isomeric and homologous forms of these compounds.

Keywords: Forensic Chemistry, Gas Chromatography, Gas Chromatography/Mass Spectrometry, Ion Trap
Application Code: Homeland Security/Forensics
Methodology Code: Mass Spectrometry
Innovations in the Analysis of Emerging Psychotropic and Synthetic Designer Drugs

Mass Spectral Tools for Characterization of Synthetic Phenethylamines

The identification of new designer drug analogs is challenging based on current gas chromatography-mass spectrometry (GC-MS) methods used in forensic laboratories. This is primarily due to the high degree of structural similarity among analogs in a given designer drug class and the lack of suitable reference standards to aid in the identification. This presentation will discuss current work in our laboratory to develop a method to associate emerging designer drug analogs according to structural classes based on data generated during their analysis.

This initial work focuses on two structural subclasses of the synthetic phenethylamines, specifically the 2C-phenethylamines and their NBOMe derivatives. The 2C-phenethylamines are 2,5-dimethoxyamphetamines with various substitutions on the aromatic ring while the NBOMe compounds are N-(2-methoxy)benzyl derivatives of the 2C-phenethylamines. Within each subclass, compounds have similar core structures but differ in the identities and positions of substituents.

A set of 2C-phenethylamines and NBOMe derivatives was analyzed by GC-high resolution MS to generate accurate mass data from which elemental formulae for dominant fragment ions were assigned. The spectral data were then probed to identify characteristic features, including the absolute and Kendrick mass defects of the molecular and characteristic fragment ions, as well as neutral losses from the molecular ion. The potential and associated limitations of each feature will be discussed and the development of a characterization scheme based on mass defects and neutral losses will be presented.

Keywords: Forensics, Forensic Chemistry, GC-MS, Time of Flight MS
Application Code: Homeland Security/Forensics
Methodology Code: Data Analysis and Manipulation
In 2014, the United Nations Office on Drugs and Crime (UNODC) reported that the number of new psychoactive substances on the global market more than doubled over the period 2009 – 2013. These abused substances include whole plant products such as the 20 species identified by the UNODC as “drugs of concern (DOC).” These plants are readily available and their use is difficult to legislate because: (1) many of the plants have alternative uses, such as in landscaping; (2) the plant foliage is not generally recognized; (3) well-established analytical methods (such as GC-MS and LC-MS) that are useful in the identification of purified or semi-purified abused substances are time consuming to perform on whole plant material and/or have not been developed for analysis of whole plant products; (4) it is impractical to develop standard protocols for analysis of the large number of plants of abuse; and (5) there is generally no statistical reporting of the level of certainty of a positive identification of a particular plant drug. We have proposed that these challenges can be overcome through the application of high throughput ambient ionization mass spectrometric methods such as direct analysis in real time mass spectrometry (DART-MS) for the analysis of whole plant material. The processing of the acquired data by multivariate statistical methods exploits the uniqueness of the characteristic mass spectral fingerprint of each plant species to yield statistical levels of probability of the plants’ identity. Furthermore, this provides the opportunity for the creation of a database of abused plants that can be used to rapidly identify plant products in a forensic context. We demonstrate the proof of principle with several of the UNODC-identified plants, including Kratom, [i]Datura[/i] spp., [i]Salvia[/i] spp., and Kava among other plants.

**Abstract Text**

In 2014, the United Nations Office on Drugs and Crime (UNODC) reported that the number of new psychoactive substances on the global market more than doubled over the period 2009 – 2013. These abused substances include whole plant products such as the 20 species identified by the UNODC as “drugs of concern (DOC).” These plants are readily available and their use is difficult to legislate because: (1) many of the plants have alternative uses, such as in landscaping; (2) the plant foliage is not generally recognized; (3) well-established analytical methods (such as GC-MS and LC-MS) that are useful in the identification of purified or semi-purified abused substances are time consuming to perform on whole plant material and/or have not been developed for analysis of whole plant products; (4) it is impractical to develop standard protocols for analysis of the large number of plants of abuse; and (5) there is generally no statistical reporting of the level of certainty of a positive identification of a particular plant drug. We have proposed that these challenges can be overcome through the application of high throughput ambient ionization mass spectrometric methods such as direct analysis in real time mass spectrometry (DART-MS) for the analysis of whole plant material. The processing of the acquired data by multivariate statistical methods exploits the uniqueness of the characteristic mass spectral fingerprint of each plant species to yield statistical levels of probability of the plants’ identity. Furthermore, this provides the opportunity for the creation of a database of abused plants that can be used to rapidly identify plant products in a forensic context. We demonstrate the proof of principle with several of the UNODC-identified plants, including Kratom, [i]Datura[/i] spp., [i]Salvia[/i] spp., and Kava among other plants.
The number of novel psychoactive substances (NPS) appearing on the illicit drug market continues to grow. These “designer” drugs are purposefully synthesized using slight modifications to known compounds to provide similar psychoactive effects while circumventing scheduling as a controlled substance. Certain derivations of known drugs are marked by enhanced potency, such as the N-benzylmethoxy derivatives of 2C-series phenethylamines, or NBOMes.

Designer drugs pose a substantial challenge in terms of identification due to their magnitude and variation, hence there is a growing need for both rapid and accurate analytical strategies. Mass spectrometry (MS) is well known for its discerning power, making portable MS systems intriguing for field-based evidence screening. When coupled to ambient ionization methods, both simplicity and throughput dramatically increases, making these systems well suited for crime scene investigation, and law enforcement.

Herein, we report the utility of a portable MS system featuring an array of “plug and play”-style ambient ionization methods in reducing the difficulty of NPS evidence screening. The rapid interchangeability of ambient MS methods allows the user to match the best processing method to the evidence at hand. To demonstrate applicability, several emerging illicit chemicals, including synthetic cathinones, synthetic cannabinoids, 2C-series phenethylamines/NBOMe derivatives, and desomorphine, were analyzed as both trace residues and bulk forensic evidence (i.e. powder-based drugs, blotter paper, etc.). The ionization sources employed, such as desorption electrospray ionization (DESI), paper spray ionization (PSI), and paper cone spray ionization (PCSI), have been shown robust towards sample complexity. Furthermore, employing automated software identification strategies based upon MS/MS spectral matching can eliminate the need for spectral interpretation by the user, allowing the use by non-technical operators.

**Keywords:** Forensics, Instrumentation, Mass Spectrometry

**Application Code:** Homeland Security/Forensics

**Methodology Code:** Mass Spectrometry
Enumerating regions and cell types in the brain has relevance for a host of developmental and disease processes, including physiologic processes and cancer. Optical chemical imaging offers a route to understanding this organization and its disruption, thereby offering opportunities to better detect, understand and diagnose disease. However, the technology must be able to rapidly image due to the need for surveying, high definition imaging or examining the chemistry in detail. Here, we first present several examples in imaging control and diseased brains from animal models of human disease. The parameters for imaging and an estimation of the complexity of data required is estimated. Next, we describe the developments in fast imaging for both infrared and Raman spectroscopy. The major goals of these approaches is to reduce scanning times by orders of magnitudes; here we show the appropriate trade-offs and scanning strategies that make it possible. Third, we present examples of spectral properties of different regions of the brain and show how these could be used for building automated, computerized recognition schemes. Finally, we demonstrate the ability to perform cytotyping and the exciting potential of the combination of fast imaging and computerized algorithms. Together, these developments present a potentially exciting route to obtaining molecular information in from brain tissues and various pathologies without the need for staining or complicated analytical measurements.

Keywords: Biomedical, Near Infrared, Neurochemistry

Application Code: Neurochemistry
Methodology Code: Near Infrared
With the advent of single cell analyses many unanticipated cell biological phenomena have been discovered including, new classes of RNA, identification of RNA targeting sequences and perhaps most surprisingly the discovery of cell-to-cell variation in transcriptome abundances even in presumptively identical cells. There is large-scale single cell RNA variability for different cell types that can’t be explained as simple molecular or technical noise. These data have led to the hypothesis that there is a many-to-one relationship between transcriptome states and a cell’s phenotype. In this relationship the functional molecular ratios of the RNA are determined by the cell systems’ stoichiometric constraints, which underdetermine the transcriptome state. Because a broad set of multi-genic combinations support a particular phenotype, changes in the transcriptome state do not necessarily lead to changes in the phenotype. By analogy cellular phenotype should not be defined based upon the expression of individual RNAs but rather as subsets of RNAs comprising selected RNA systems where the system-associated RNAs are balanced with each other to produce the associated cellular function. Data in support of these ideas, will be presented from live mouse and human cells, fixed tissue analysis and subcellular regional analysis. These ideas provide a framework for understanding cellular heterogeneity in phenotypic response to variant conditions. Further they provide a means for rethinking how to manipulate cellular responses (using novel functional genomics methodologies) so that desired cell homeostatic states or physiological outcomes are obtained.

Keywords: Biological Samples, Genomics, Method Development, Microscopy

Application Code: Genomics, Proteomics and Other ‘Omis

Methodology Code: Microscopy
Comprehensive characterization of signaling peptides in a nervous system is often critical to deciphering the functionality of a neural circuit yet it presents a daunting challenge due to low level of these signaling molecules present in highly complex biological matrices. To address technical challenges of de novo sequencing of neuropeptides, we employed isotopic formaldehyde labeling to improve fragmentation patterns for unambiguous assignment of sequence-specific product ions. Furthermore, we incorporate this labeling method followed by electron-transfer dissociation (ETD) fragmentation and ion mobility spectrometry (IMS) to study the underlying mechanism of resulting simplification of gas-phase tandem MS fragmentation.

In addition to large-scale de novo sequencing for discovery of novel neuropeptides, multiplex quantitation based on isobaric tagging reagents represent a new direction for high-throughput quantitative peptidomics/proteomics. Towards this end we developed novel tandem mass tagging reagents based on dimethylated amino acids and employed these new reagents to produce differential display of neuropeptidomes under different physiological conditions such as feeding and hypoxia. A potential limitation to the current isobaric tagging for quantitation is the underestimation of fold-changes due to precursor isolation interference. We propose and demonstrate novel use of IMS to separate co-isolated precursor ions in the ion mobility drift cell followed by tandem MS fragmentation. This new strategy improves peptide identification and quantitation accuracy using isobaric tandem mass tags. Furthermore, using ion mobility to separate tandem MS-generated epimeric fragment ions we developed a novel site-specific strategy to rapidly and precisely localize D-amino acids in peptides, a unique post-translational modification (PTM) occurring in neuropeptides.

**Keywords:** Mass Spectrometry, Neurochemistry, Peptides, Tandem Mass Spec

**Application Code:** Neurochemistry

**Methodology Code:** Mass Spectrometry
Numerous small molecules including amino acids, amino acid derivatives, and peptides have been identified as chemical neurotransmitters. Neuronal cells using different neurotransmitters have spatially distinct patterns that translate into highly heterogeneous chemistries in brain extracellular microenvironments. Accordingly, measurements of neurotransmitters with high spatial, temporal, and chemical resolution will prove fundamental to understanding basic neural information processing. Identifying artificial neurotransmitter receptors from synthesized combinatorial libraries is key to advancing chemical selectivity for neurotransmitter sensing in vivo. We have developed a number of avenues for controlled placement and ligation of small molecules on nanoengineered substrates that enable selection of molecular recognition elements. We discovered methods of patterning small-molecule substrates with features as small as tens of nanometers and patterns over millimeters. As such, we have identified a set of design rules that achieve high degrees of specific and selective biomolecule recognition of immobilized small molecules. Recent advances include identification of surface-patterned neurotransmitters by nucleic acid aptamers, highly optimized substrate-mediated DNA hybridization, and field-effect transistor electronic neurotransmitter sensing. Small-molecule-functionalized nanomaterials are generalizable for a wide range of biosensing and bioengineering applications.
In the postgenomic era, one expects the suite of chemical players in a brain region to be known and their functions uncovered. However, many cell-to-cell signaling molecules remain poorly characterized and for those that are known, their localization and dynamics are oftentimes unknown. A suite of small-scale bioanalytical approaches are described that allow the investigation of individual neurons and small brain regions; these approaches include capillary scale separations, direct mass spectrometric-based profiling and mass spectrometry imaging. A key to successful measurement involves optimized tissue and cell sampling protocols. Depending on the sample being assayed and metabolites being measured, we use mechanical isolation, optical tweezers, patch pipettes, dialysis probes and microfluidics, all of which have advantages for specific sample types. Several applications of single cell microanalysis are highlighted including the discovery of unusual metabolites to characterizing the peptides in single cells. Single cell assays allow differences in the metabolomics and peptidomics from supposedly homogeneous populations of cells to be explored. As a further example, a unique matrix assisted laser desorption / ionization time of flight mass spectrometry approach is used to probe thousands of endocrine cells for their neuropeptide content. Current technology efforts involve extending the depth of metabolome coverage and adapting our approaches to high throughput single cell assays. By obtaining information from tens of thousands of individual cells, rare cells are found and subtle differences in cell populations can be measured. Imaging mass spectrometry and dynamic sampling of the extracellular environment also provide a functional context for the discovery of novel cell to cell signaling molecules. Our overarching goal is to uncover the complex chemical mosaic of the brain and pinpoint key cellular players in physiological and pathological processes.

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

**Application Code:** Neurochemistry

**Methodology Code:** Mass Spectrometry
Nanotechnology and Bioanalytical Chemistry

**Surface Modification with Functional Molecular Patterns Revealed by In-Situ STM**

For biochemical research and bio-device fabrication, modifying a solid surface by molecular patterns, adsorption and regular dispersion of biomolecules are important issues, which can be realized by nanoscience and nanotechnology, especially by molecular engineering [1]. As a result of intensive study, self-assembly is a promising strategy for modifying and constructing molecular nanostructures on a surface. A challenge in self-assembly is the precise control of nanostructure and arrangement of molecules. In this presentation, molecular structures on different substrates such as Au(111) and HOPG are reported. The structures are investigated by scanning tunneling microscopy (STM) in ambient and electrolyte solution. The adsorbed molecules can form well-defined molecular assembly with different symmetries. The assembly with the individual molecules could also be polymerized into molecular nanowires and nanonetworks, schematically shown in the attached figure [2-4]. Biomolecules can be deposit in the cavity of the network. The modified surface shows potential in biomolecule research and bio-device fabrication.

References:

**Keywords:** Electrochemistry, Imaging, Single Molecule, Surface Analysis

**Application Code:** Nanotechnology

**Methodology Code:** Microscopy
Nanotechnology and Bioanalytical Chemistry

Organometallic Single-Ion Magnets

After the milestone discovery of the first single-molecule magnets (SMMs) Mn12ac, many new SMMs were structurally and magnetically characterized. The most studied systems are mainly conventional coordination compounds with polynuclear structures. From 2011, we explored a series mononuclear organometallic sandwich molecules, Cp*LnCOT (Cp* = C5Me5-, pentamethylcyclopenta-dienide; COT = C8H82-, cyclooctatetraenide; Ln = Dy3+, Ho3+, Er3+), which behave as a single-ion magnets (SIMs) [1-3]. It opened a door of SMMs to the chemists in organometallic chemistry. Recently, we found that some new sandwich and half-sandwich lanthanide organometallic molecules could also show the slow relaxation of magnetization [4-7]. Moreover, a few new linear two-coordinated Co(I)/Co(II) organometallic SIM were discovered [8]. We hope these systems can provide new understandings of slow magnetic relaxation and new clues on the design and synthesis of molecular nanomagnets.

References

Keywords: Luminescence, Materials Characterization, Nanotechnology, X-ray Diffraction

Application Code: Nanotechnology

Methodology Code: Physical Measurements
Abstract Text

Metalloproteins are one of the most diverse species of proteins in cells which contain at least one specific metal ion for either functionalization purpose or structural stability. It accounts for at least one third of proteome, which however are largely uncharacterized. Development of robust methods and platform which could be applied to map the metalloproteins in proteome-wide of certain cell type or tissue is current a challenge for analytical chemistry. We recently developed a method for identification of metalloproteins based on the separation of continuous-flow gel electrophoresis coupling to ICP-MS detection. It was successfully applied to profile metalloproteins in bacteria and blood samples, demonstrating its potential in mining of metalloproteins in biological samples.

3D printing technology for the biomedical applications expand rapidly, including printing tangible models for surgical preparation, printing tissue scaffolds and organs, and even succeeded printing blood vessels. It has also been applied in the area of analytical chemistry for its advantages such as flexibility, high resolution, high building speed and personalization.

Recently, we redesigned the GE-ICP-MS system and fabricated the core separating device, interface coupling to ICP-MS and related parts via 3D printing, to improve the performance on analysis of metalloproteins. The printed system showed improved performance in the analysis of metalloproteins, i.e., reduced dead volume, increased separation resolution, increased sample transferring efficiency, et al. Amazingly, the total cost of the printed system was less than five hundreds of RMB Yuan, which accounted for only around 1% cost of previous corresponding set-up. 3D printing not only provide a convenient way to fabricate comprehensive devices precisely in lab, also dramatically reduced the cost to set up them.

Keywords: Electrophoresis, ICP-MS, Protein
Application Code: Bioanalytical
Methodology Code: Mass Spectrometry
The synthesis and the translational control of new proteins are necessary for many intricate biological processes, such as cell growth, differentiation, metabolism, and migration. Recently, great progresses have been achieved on adopting a powerful technique named bioorthogonal noncanonical amino acid tagging (BONCAT) coupled with click chemistry to visualize newly synthesized proteins. In my group, we have developed several methods to study the new proteins in cells. For example, we used the nano-sized metal oxides to replace copper(I) ions as catalysts so as to reduce the cytotoxicity. We also used super-resolution fluorescence microscopy to study the newly synthesized proteins within subcellular distribution after drug stimulation. During our study, we found that BONCAT method is unable to visualize a specific protein of interest (POI) as newly synthesized in situ. Therefore, we develop a FRET-based strategy for imaging the newly synthesized endogenous POI within cells. We found that photobleaching method and FLIM-FRET show the distribution of newly synthesized proteins more accurately compared to direct observation of FRET signals, thus making them most suitable for our FRET strategy. We demonstrated the capability of this FRET imaging method by visualizing several newly synthesized proteins including tubulin, TDP-43 and CaMKII in different cell lines. This study may provide new insights into tracking POI synthesis and redistribution at different intervals, in their native cellular context.

Keywords: Biological Samples, Fluorescence, Imaging
Application Code: Biomedical
Methodology Code: Fluorescence/Luminescence
Single-molecule fluorescence imaging of protein dynamics at the nanoscale is becoming a powerful tool for the real-time study of protein activation and cellular transportation to achieve a better understanding of cell signal transduction mechanism. Based on our single-molecule imaging and quantitative analysis of membrane receptor stoichiometry and dimerization kinetics on cell surface, we further monitored the endocytosis and intracellular trafficking of transforming growth factor \( \alpha \) (TGF-\( \alpha \)) receptors. Two major endocytic pathways, clathrin- and caveolae-mediated endocytosis, have been reported to independently mediate the internalization of TGF-\( \alpha \) receptors, leading to the signal up-regulation and down-regulation respectively. We achieved real-time observation of both caveolae- and clathrin-mediated TGF-\( \alpha \) receptor endocytosis, and demonstrated that the two endocytic pathways can be fused during TGF-\( \alpha \) receptor endocytic trafficking. This generates the new multifunctional sorting device, caveolin-1-positive early endosomes, for TGF-\( \alpha \) receptors activation and recycling. We have also investigated and quantified the membrane docking dynamics of individual downstream protein Smad3. It was found that intracellular Smad3 molecules docked to cell membrane in both unstimulated and TGF-\( \alpha \) stimulated cells, but with different diffusion rates and dissociation kinetics. The change in its membrane docking dynamics has been used to study the activation of Smad3 to propagate TGF-\( \alpha \) signals from cell membrane to nucleus.

**Keywords:** Bioanalytical, Fluorescence, Monitoring, Quantitative

**Application Code:** Bioanalytical

**Methodology Code:** Microscopy
Organs-on-chips are as diverse as the biological models they are meant to study. “Bottom-up” cell culture models involve seeding cells into a chip to form 2D or 3D tissue-like structures, whereas “top-down” biological approaches use organotypic tissue samples directly obtained from mammals or human subjects. This presentation will focus on examples from our labs in which organ-chips incorporate means to both monitor culture conditions and quantify cell behaviour. In our “bottom-up” example, we present a new approach for real-time, non-invasive, label-free monitoring of cellular micromotion in endothelial cell cultures. Cells are cultivated in a microcuvette formed by etching through the silica and waveguiding layers of a solid-state device. Light (638 nm) is directly coupled into the cell monolayer from the integrated waveguide, and forward-scattered light is recorded as a measure for cellular micromotion. Motion behaviour could serve as a readout for changes in the cytoskeleton caused by various chemical and physical factors. Observation of cytoskeletal changes indicative of inflammatory status using this approach could thus prove useful for studying the role of endothelial cells in the onset of cardiovascular diseases. In a “top-down” example, we report what we believe is the first example of an oxygen consumption experiment with perifused tissue slices in a controlled microfluidic environment. A microfluidic device for perfusion culture of precision-cut liver slices was micromilled in polycarbonate with slots for the insertion of microsensors to monitor oxygen consumption of the slice. Deliberate changes in medium composition induced variations in slice metabolism which could be correlated to altered oxygen levels recorded after the slice in the device. Incorporation of oxygen microsensors thus allows not only enhanced control of the incubation environment, but also the acquisition of additional physiological information directly in-line and in real time.

Keywords: Biological Samples, Biomedical, Lab-on-a-Chip/Microfluidics, Pharmaceutical
Application Code: Pharmaceutical
Methodology Code: Microfluidics/Lab-on-a-Chip
Our lab has long been interested in the development of robust microfluidic devices that integrate cell culture and analysis. This includes the integration of PC 12 cell culture with microchip based analysis (to monitor stimulated exocytosis of multiple neurotransmitters) as well as creation of an endothelium mimic with integrated electrodes for nitric oxide detection. This talk will detail recent technology we have developed around these applications. This includes the fabrication and use of polystyrene-based microfluidic devices, where encapsulation of materials (tubing and electrodes) can be used to improve cell viability and device performance. We will show that a unique feature of this encapsulation approach is the ability to create planar membranes from 3-dimensional pillar electrodes. Work towards using this type of device to monitor cell-to-cell interactions will be discussed. Finally, more recent work on the development of more realistic, 3-dimensional cell culture models for microfluidics will be presented. This includes the use of 3D-printing to create an air sheath device to dynamically focus electrospun fibers into a fully closed fluidic channel, with a fibrous scaffold being created on the inner channel wall. Fibroblasts and macrophages were used to test cell compatibility of the scaffold, the results of which showed that viable cells with the expected morphology and size can be cultured directly on the spun fibers. We will discuss these results and how electrospinning can be used to easily and quickly create 3D scaffolds that can improve the culture conditions in microfluidic devices.

Keywords: Electrophoresis, Lab-on-a-Chip/Microfluidics, Microelectrode, Separation Sciences
Application Code: Bioanalytical
Methodology Code: Microfluidics/Lab-on-a-Chip

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Living systems are dynamic. The same drug, hormone, or signaling molecule can have different effects depending on dosing regimen and secretion profile. Conventional cell cultures, however, are static and have difficulty recreating physiologic pharmacokinetic profiles or biorhythms. This presentation will describe some work from our lab to fill this gap with microfluidic cell culture systems. In one example, using a kidney-on-a-chip, we compare nephrotoxicity of gentamicin, administered at the same total dosage, but using two different 24-hour regimens that mimic the pharmacokinetics of bolus injection or continuous infusion in humans. The perfusion culture utilized is important both for controlling drug exposure and for providing cells with physiological shear stress. The bolus injection regimen attenuated epithelial barrier dysfunction and cytotoxicity more than continuous infusion regimen. In another example, we use microfluidic pulsatile stimulation to dissect a G-protein coupled receptor (GPCR) signaling pathway architecture (M3 muscarinic receptor) and show that maximum transcription factor (NFAT) activation is achieved when using a pulsed stimulation rather than continuous stimulation due to reduced receptor desensitization. Importantly, the results demonstrated the general potential of using microfluidic cell culture models for pharmacokinetics, toxicity, and signaling pathway analysis studies.
A key step in the drug development process is the pre-clinical stage, where the pharmacokinetic (PK) profile of a drug candidate molecule is determined. In vivo animal studies, while providing a living system comparable to that of humans, consume large quantities of materials and time, are highly technical, and the obtained data does not often translate to human scale and metabolism. Recently, there has been interest in further developing in vitro systems for use in tandem with current in vivo methodology to increase efficiency of study design while reducing material costs. While in vitro PK profiles will never be able to incorporate all of the variables present in an in vivo model, nor the pharmacodynamic (PD) response, recent reviews and reports in the literature clearly demonstrate the need for improved in vitro models for PK/PD studies. Here, we describe multiple versions of 3D printed fluidic devices capable of generating dynamic, in vitro PK profiles that also enable direct access to cells for measurement of cellular PDs. These devices utilize disposable Transwell® inserts whose bottoms are comprised of a porous semipermeable membrane that separates a flowing stream of drug-containing buffer in the fluidic channels from a standard-sized microplate well. These fluidic devices provide a rigid, reusable platform, whereupon seeding specific cell types in the disposable cell culture inserts create biomimetic system. In this presentation, we will also demonstrate how such systems may be useful as a screening system for antibiotic efficacy against bacterial infections.
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 single cell analysis. In this paper, the use of microchip electrophoresis (ME) for the determination of reactive oxygen and nitrogen species (RNOS) in cell lysates will be described. A method has been developed to simultaneously measure superoxide and nitric oxide using MitoSOX and DAF-FM and microchip electrophoresis with fluorescence detection in macrophage lysates. The use of HK-Green is also being evaluated for peroxynitrite detection. ME makes it possible to separate the different fluorescent RNOS products from interferences and side products. However, the ME-FL method provides only an integrated amount of RNOS produced during the incubation time. In order to determine the instantaneous concentrations of RNOS, microchip electrophoresis with electrochemical detection is being evaluated. With this direct detection method, the instantaneous concentrations of nitrite, peroxynitrite, and nitric oxide can be determined. Progress toward a dual electrochemical/fluorescence detection system for single cell cytometry will also be discussed.

Keywords: Biological Samples, Electrochemistry, Fluorescence, Lab-on-a-Chip/Microfluidics

Application Code: Bioanalytical

Methodology Code: Microfluidics/Lab-on-a-Chip
Mass cytometry is a powerful technique for the high throughput multiparameter analysis of biomarker expression on single cells. Recently the scope of mass cytometry has been expanded through the use of laser ablation to enable imaging mass cytometry of histological sections. We are interested in the development of metal chelating polymers (MCPs) and lanthanide nanoparticles for attachment to antibodies to serve as reagents for these techniques. MCP-Ab conjugates that carry ca. 200 copies of a metal isotope enable detection of biomarkers expressed at relatively high levels (5000 to $10^7$ per cell). There is a need to detect biomarkers present at much lower levels of expression. Antibody-nanoparticle (Ab-NP) conjugates can carry 10,000 or more copies of a metal isotope, offering the possibility of two orders of magnitude greater sensitivity. For this purpose, we have synthesized NaLnF$_4$ NPs with diameters ranging from 5 nm to 20 nm. One challenge is to control the size and to obtain these NPs with as narrow as possible size distribution. A second challenge is to coat the NP surface to provide colloidal stability in aqueous buffers compatible with mass cytometry as well as functionality to serve as sites for Ab attachment. The biggest challenge is to find surface coatings for the NPs that will minimize or suppress non-specific interaction or engulfment by cells, so that the background signal of cells lacking a targeted antigen will remain suitable low. My talk will present our progress in meeting these challenges.

Keywords: Bioanalytical, Immunoassay, Mass Spectrometry
Application Code: Bioanalytical
Methodology Code: Mass Spectrometry
Recent Developments in Mass Cytometry

Multidimensional Profiling Using Mass Cytometry (CyTOF) Reveals Individual Variation in Immune Responses

Mass cytometry or CyTOF (Cytometry by Time-Of-Flight) is a new technology for real time multiparameter (>42) single cell analysis providing unprecedented multidimensional cellular and proteomic analyses. An important advantage of CyTOF over fluorescence activated flow cytometry is the use of metal-conjugated antibody labels and detection by mass spectroscopy, thus there is no overlap between channels and little/no background, both challenges for immunologists. CyTOF provides excellent sensitivity for immune cell profiling from as few as 1000-10,000 peripheral blood mononuclear cells, a critical advantage as sample quantities are frequently limiting. CyTOF provides high quality dynamic characterization of signaling pathways to define mechanisms of cellular regulation, providing excellent support in translational studies.

We have used high-dimensional mass cytometry to investigate cellular phenotypes in primary cells from peripheral blood, skin, and airway inflammatory cells. We quantified cellular frequency and functional status of defined cell subsets in asthma and cystic fibrosis. We have employed CyTOF to define immune responses to West Nile virus, an emerging mosquito-borne disease that can lead to severe neurological illness, especially in the elderly. We investigated susceptibility- and age-related differences in cell phenotype and functional responses to virus in Natural Killer (NK) cells using a customized antibody panel that detects 23 surface receptors and 10 functional markers in each cell simultaneously. Through this unbiased approach, we distinguished immunological and chronological age and identified phenotypic clusters of NK cells with significant differences in combinatorial expression of activating and inhibitory NK receptors that may have implications for viral responsiveness. Thus, CyTOF provides unprecedented multidimensional detail of phenotypic and functional parameters of immune subsets and supports our ongoing studies to address detailed mechanisms of immune responses in multiple human populations.

Keywords: Biomedical, Mass Spectrometry
Application Code: Biomedical
Methodology Code: Mass Spectrometry
Recent Developments in Mass Cytometry

Mass Cytometry for the Quantification of Autophagy in Skeletal Muscle Cell Sub-Populations

It has become increasingly apparent that meaningful understanding of cellular processes – from normal development to disease progression – is more informative when studies achieve single cell resolution. Until recently, a high-throughput, sensitive, and highly-multiplexed technique was not available. Mass cytometry is an emerging technology that measures cellular markers on the surface or within individual cells that allows for detection and quantification of upwards of 45 separate cellular targets.

Skeletal muscle is a complex tissue, comprised of many cell types that contribute to its overall health and regenerative capacity, that will benefit from the highly multiplexed analysis such as is possible with mass cytometry. It has shown that skeletal muscle mass and function decline with age. Dysregulation of macroautophagy, the biological process by which damaged or long-lived organelles and proteins are recycled, has been implicated as playing a key role in this age-related decline. We have developed complimentary panels of antibodies to simultaneously categorize cell sub-populations based on phenotypic identity, and quantify autophagy-related proteins in individual cells. This approach facilitates unprecedented insight into how dysregulated autophagy in specific cell populations (i.e. regenerative, immune and contractile) contribute to the decline of muscle mass and function with age.

Two mass cytometry panels have been developed: The phenotypic panel demonstrated the categorization of 8 sub-populations of cells using 16 antibodies in a murine model of skeletal muscle. The autophagy panel used 16 antibodies and is able of detecting changes in autophagic function in vitro, using an ATG5 knock-down myoblast cell line exhibiting altered autophagy, and in vivo, using young and aged murine models of skeletal muscle.

Abstract Text

Skeletal muscle is a complex tissue, comprised of many cell types that contribute to its overall health and regenerative capacity, that will benefit from the highly multiplexed analysis such as is possible with mass cytometry. It has shown that skeletal muscle mass and function decline with age. Dysregulation of macroautophagy, the biological process by which damaged or long-lived organelles and proteins are recycled, has been implicated as playing a key role in this age-related decline. We have developed complimentary panels of antibodies to simultaneously categorize cell sub-populations based on phenotypic identity, and quantify autophagy-related proteins in individual cells. This approach facilitates unprecedented insight into how dysregulated autophagy in specific cell populations (i.e. regenerative, immune and contractile) contribute to the decline of muscle mass and function with age.

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Keywords: Biological Samples, Biotechnology, ICP-MS, Informatics
Application Code: Biomedical
Methodology Code: Mass Spectrometry
Identifying the molecular mechanisms that control the progression of cell differentiation and the branch points of cell fate is an important goal for stem cell biology. High-content single-cell analysis methods such as flow cytometry, mass cytometry, and single-cell RNA-seq have proven useful for identifying and molecularly characterizing rare and transitory cell populations, but using this information to reconstruct cell lineage hierarchies remains challenging. FLOW-MAP is a graph-based, force-directed layout algorithm that was developed to serve this need by tracking cell populations as they change over time within single-cell datasets, and combining these trajectories into a global network graph that can be used to infer lineage relationships between cell types. To investigate the molecular mechanisms that drive cell fate decisions during germ layer formation, this approach was applied to embryoid body differentiation of 2i/LIF-cultured mESCs in conditions that favor ectoderm, mesoderm, or endoderm formation. Every 24 hours from Day 0 to Day 11, cell samples were collected with rapid dissociation followed by immediate paraformaldehyde fixation. Single-cell mass cytometry analysis of these samples followed by force-directed layout analysis allowed for molecular characterization of the intermediate cell types along these differentiation trajectories, and the construction of a global lineage hierarchy map for germ layer formation. In addition to in vitro pluripotent stem cell differentiation, this single-cell time course-based analysis strategy can be applied to other dynamic cell systems in vitro and in vivo, including direct reprogramming, embryonic development, immune response, oncogenesis, and drug resistance.

Keywords: Biomedical, Data Analysis, Mass Spectrometry, Stem Cell
Application Code: Biomedical
Methodology Code: Data Analysis and Manipulation
Recently, there have been significant advances in single-cell genomic and proteomic technologies that can measure the expression of thousands of mRNA transcripts and dozens of proteins. However, this data suffers from sparsity and noise. Furthermore, its high dimensionality makes interpreting the data difficult for biologists. Our aim is to facilitate interpretation by providing a set of tools and novel algorithms that allow biologists to extract meaningful and predictive information from the data, and which yield clear and concise visualizations. In my lab we utilize a diffusion framework to learn the manifold geometry of the data. This framework models the local affinity between high-dimensional data points using a kernel function and then utilizes graph diffusion to form long range connections and paths through the data. Our framework has used to impute and correct noisy single-cell RNA-sequencing data, using a method called MAGIC that utilizes the Markov diffusion operator that is part of the framework that models cellular neighborhoods. Then we extend this method to a family of novel transformations and algorithms performed on the Markov diffusion operator in order to emphasize several types of patterns in the data.

Keywords: Bioanalytical, Genomics
Application Code: Bioanalytical
Methodology Code: Laboratory Informatics
Cultured neurons are widely used and relied upon for drug screens, toxicity testing and basic research. Yet, there remain unmet needs to scale up neuron-based screens and develop models that accurately reflect the in vivo neuronal environment and proper development and function of neuronal networks. Current high-throughput screening methodologies are optimized for dividing cells that can be expanded to generate large sample sizes, not for post-mitotic cells such as neurons. Neurons also exhibit unique polarized morphology that informs their function and response to damage. Neuronal networks require long culture times and high cell densities to develop functional synaptic connections. Scalable neuron-based assays are an emerging focus area in part due to the increased availability of human induced pluripotent stem cells (hiPSCs) used to generate cultured neurons from patients, providing unprecedented access to human neurons for drug and toxicity screens. This symposium will focus on the latest developments to overcome challenges in neuron-based screening, including the use of 3D neuronal networks, novel scalable mini-culture platforms, neurovascular unit models, and innovative scalable assays for synaptic development and function.
Neuronal cultures are challenging to generate and sustain in vitro, but critical for the assessment of the impact of drugs, mechanical damage, toxicology, sensory functions and many other outcomes. Neuronal cultures must provide many different features, tailored to the particular experimental goal, in order to achieve relevant physiological measures. Such features can include nerve cell type, cocultures with other cell types, a matrix environment to support the cells in 2D or 3D, and cultivation time to assess acute or chronic impacts on function. Towards these needs, we have developed a series of 3D neuronal-based tissue culture systems to capture physiologically-relevant system functions for the study of nerve cell responses. These systems include 3D cortical brain tissues and a variety of innervated tissue-specific systems such as the cornea and skin. The approaches utilized to generate these nerve-containing tissues and results from these systems will be reviewed.
One of the primary limitations in drug discovery and toxicology research is the lack of good model systems between the single cell level and animal or human systems. This is especially true for neurodegenerative diseases such as ALS, Alzheimer’s, and spinal cord injury. In addition, with the banning of animals for toxicology testing in many industries body-on-a-chip systems to replace animals with human mimics is essential for product development and safety testing. Our research focus is on the establishment of functional in vitro systems to address this deficit where we have created organs and subsystems to model motor control, muscle function, myelination, blood brain barrier function as well as cognitive function. The idea is to integrate microsystems fabrication technology and surface modifications with cellular components, for initiating and maintaining growth into biologically, mechanically and electronically interactive functional multi-component systems. Our advances in culturing adult rat, mouse and human mammalian spinal cord, hippocampal neurons, muscle and endothelial cells in a defined serum-free medium, suggest excellent potential for answering questions related to neuronal maturation, aging, neurodegeneration and injury. We are using this ability to manipulate the biological systems and integrate it with silicon-based systems to create cell-based sensors for high content drug discovery. Examples will be given of some of the more advanced CNS and PNS body-on-a-chip systems being developed as well as the results of five workshops held at NIH to explore what is needed for validation and qualification of these systems by the FDA and EMA.

Keywords: Clinical/Toxicology, Drug Discovery, Lab-on-a-Chip/Microfluidics, Neurochemistry

Application Code: Drug Discovery

Methodology Code: Microfluidics/Lab-on-a-Chip
Age is the biggest risk factor for the major adult-onset neurodegenerative disorders, including Alzheimer’s disease and Parkinson’s disease. Populations worldwide are aging rapidly so the incidence of these diseases is rising, and yet, no significant disease-modifying therapies are available for any of them. The conventional preclinical pipeline has failed to accurately predict the results of clinical trials, and the risk involved in pursuing neurotherapeutics has limited investment by the pharmaceutical industry dimming prospects further. We developed a platform to do high-throughput (HT) longitudinal single-cell analysis called robotic microscopy that is orders of magnitude more sensitive than commercially available HT screening systems. The sensitivity comes from the ability of the system to identify and track individual live cells as often and for as long as the investigator wishes, which makes it possible to analyze the data using statistical tools similar to those used in clinical trials. The added sensitivity makes it feasible for us to use primary neurons and differentiated induced pluripotent stem cells as a small molecule and genetic screening platform. Fewer cells are needed to achieve the necessary power for the study, making it more affordable, and the single-cell analysis approach is well suited to deal with the cellular heterogeneity inherent to primary culture. To expand the scope and depth of the phenotypic screening platform, we are developing an array of more than 270 biosensors called the “physical exam of the cell.” With these tools, we hope to extend our phenotypic screening platform from genetic causes of neurodegenerative diseases and mental illnesses to more common forms.

Keywords: Drug Discovery, Imaging, Robotics, Statistical Data Analysis
Application Code: Drug Discovery
Methodology Code: Molecular Spectroscopy
Characterization of the potential adverse effects is lacking for tens of thousands of chemicals that are present in the environment, and characterization of developmental neurotoxicity (DNT) hazard lags behind that of other adverse outcomes (e.g. hepatotoxicity). This is due in part to the high cost and large number of animals needed to characterize DNT hazard. Thus, faster, less expensive approaches for DNT testing are needed. To address this need, EPA scientists have been developing assays capable of screening and prioritizing chemicals for DNT hazard. These assays use high-content imaging (HCI) and microelectrode array (MEA) recording to develop cell-based assays that detect chemical effects on key neurodevelopmental processes, including proliferation, differentiation, synaptogenesis and network function. The ability of these assays to identify compounds with DNT hazard is being evaluated by testing a reference set of developmentally neurotoxic compounds and by developing mathematical approaches to unbiased compound prioritization. The assays developed to date are able to detect effects of reference compounds with high sensitivity and specificity, and now are being used to evaluate compound sets that are of interest to regulatory decision-makers. This presentation will provide an overview of the various assays and examples of how they currently providing information to regulatory decision-makers. (This work was supported by the US Environmental Protection Agency. This abstract does not reflect EPA policy).

Keywords: Clinical/Toxicology, Data Analysis, Drug Discovery, Microelectrode
Application Code: Drug Discovery
Methodology Code: Physical Measurements
Wearable and Point-of-Care Sensor Technologies for Biomonitoring

Wearable Sweat Sensors

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 advancement 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.

Keywords: Electrochemistry, Semiconductor, Sensors
Application Code: Biomedical
Methodology Code: Sensors
The need to monitor and detect biological elements, related to human and environment health in a fast and reliable way, is one of the challenges faced by humanity at the dawn of the 21st century. Tests done nowadays in laboratories (as ELISA, PCRs, cell cultures, etc.) are slow (from several hours to days) and expensive. Modern diagnostics is demanding novel analytical tools that could enable quick, accurate, sensitive, reliable and cost-effective results so that appropriate treatments can be implemented in time, leading to improved outcomes. Such portable point-of-care (POC) devices, able to deliver an instant diagnostics, could become a reality soon thanks to the last advances in nanobiosensors, lab-on-a-chip, wireless and smart-phone technologies which promise to surpass the existing challenges, opening the door to a global diagnostics access.

The driving force of our research is to achieve such ultrasensitive platforms for POC label-free analysis using nanophotonic technologies and custom-designed biofunctionalization protocols, accomplishing the requirements of disposability and portability. We are using innovative designs of nanophotonic biosensors based on silicon photonics technology (bimodal waveguide nanointerferometers) and full microfluidics lab-on-chip integration.

We have demonstrated the suitability of our photonic nanobiosensors for the clinical diagnostics, with extremely sensitivity and selectivity and directly using untreated human fluids, as for the evaluation of infectious microorganisms (at few cfu/mL) in human ascetic fluids, the early detection of colorectal cancer though the presence of autoantibodies in patients serum or the detection of microRNA biomarkers at aM level related to cancer progression in urine or serum, among others.

Keywords: Bioanalytical, Biosensors, Nanotechnology, Sensors
Application Code: Bioanalytical
Methodology Code: Sensors
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.

Keywords: Electrochemistry, Metals, Sensors
Application Code: Biomedical
Methodology Code: Electrochemistry
Accurately assessing children’s exposure to ultrafine particles (UFP) is difficult due to the high spatiotemporal variability of UFP and the time-activity patterns of children. To address the need for personal monitoring, a novel sensor for measuring UFP exposure with high spatiotemporal accuracy has been developed. The objective of this study was to conduct a field test of this sensor on asthmatic children. Children ages 8-11 with asthma wore the sensor at school, during transit periods between school and home, and in their home for 2-4 hours on 2 consecutive days. The sensor recorded UFP number concentration at one second intervals and GPS location allowing for comparisons of UFP exposure at homes, schools, and during transit. A mixed-effects linear model was used to compare UFP concentrations across each microenvironment. The overall total median personal exposure to UFP was 12,900 particles / cm³ (p/cc); median UFP exposure at homes, schools, and during transit was 17,800, 11,900, and 13,600 p/cc, respectively. Riding in a car (36% increase) and walking (251% increase) were associated with significantly elevated UFP exposure compared to home concentrations. Results from the field test indicate the personal sensor can measure real-time exposure to UFP with high spatiotemporal resolution. Future research will include sensor modifications to reduce its size, noise, and weight. Additional personal sampling is planned to assess the impact of short-term and peak UFP exposure on lung function.
Biodegradable Mg offers advantages over permanent stainless steel for implants that are used for broken bone repair. Mg alloys gradually dissolve, avoiding the need for removal by a later surgery if complications arise. The biodegradation of magnesium and its alloys can be monitored by measuring the H[\textsubscript{2}] evolved. Hydrogen has proven to be especially useful for monitoring Mg biodegradation during \textit{in vivo} evaluation with test animals because very sensitive and selective sensors are available. A visual H[\textsubscript{2}] sensor consisting of a thin film of H[\textsubscript{2}]-sensitive material (MoO\textsubscript{3} and Pd catalyst) coated on a flexible plastic sheet has been demonstrated for this application. The sensor can be pressed against the skin directly above a biodegrading implant where it responds to H[\textsubscript{2}] levels permeating through the skin. Although the H[\textsubscript{2}] levels are very low, the sensor changes color to give a three dimensional (3D) visualization of H[\textsubscript{2}] permeation through the skin. Monitoring H[\textsubscript{2}] transdermally by a simple, non-invasive procedure with a sensor provides an effective means for monitoring Mg biodegradation in point of care applications.

Keywords: Bioanalytical, Biological Samples, Sensors, UV-VIS Absorbance/Luminescence
Reference materials are a critical component of any analytical testing laboratory. They become even more valuable when the laboratory is working with dietary supplements and natural products. The analysis of dietary supplements often requires the use of new and novel test methods. We do not have nearly as many official compendium test methods for dietary supplements as we do with foods and food products. This situation often results in the needs for method modification and method development. Whenever a laboratory is modifying existing methods, or validating new methods – reference materials are a critical tool that is required to evaluate the success of the projects. Dietary supplements are often very complex products with numerous active ingredients. A multi-vitamin multi-mineral tablet can contain as many as 35 active compounds. In addition to vitamins and minerals, dietary supplement often contain active materials from plants and plant extracts. In order to effectively monitor test methods in a large analytical laboratory, reference materials are utilized on an almost daily basis.

Reference materials play a critical role with the analysis of dietary supplements when used for routine analysis – as well as for method development and method modification studies. These tools are essential for effective quality control and evaluation of method accuracy.

Keywords: Analysis, Laboratory, Reference Material, Sample & Data Management
The regulatory climate in the dietary supplements industry has heated up recently in the wake of enforcement actions by State Attorneys General, as well as some negative media attention; many companies are allocating higher budgets to Quality Control departments and expanding testing programs. As business models increasingly trend toward being less vertically integrated, diversification and globalization of the botanical supply chain has led to significant challenges in assuring quality products for consumers. The language in the Code of Federal Regulations (21 CFR part 111) is subject to differing interpretations around establishing appropriate identity and potency test procedures. There is a need for well characterized reference materials and efficient broadly applicable standard analytical methods. The complex nature of botanical dietary supplements and the proliferation of myriad finished product formulations present challenges in regard to fit-for-purpose scientifically valid methods for each unique matrix. Many standard and compendial methods have a limited scope and are validated for single botanicals. As the expectation of transparency and the role of Quality Control testing in the supplements world evolve, can analytical chemists come to consensus on how to reconcile the desire for analytical excellence with the business needs of the industry?

Keywords: Method Development, Natural Products, Quality, Validation
Application Code: Quality/QA/QC
Methodology Code: Liquid Chromatography
Analytical Methods and Reference Materials for Dietary Supplements

Spectral Correlation Method for Verifying the Presence of Botanical Ingredients in Supplements

The chemical composition of a botanical ingredient is dramatically changed during the production of a commercial supplement. In general, processing requires an extraction, usually a back extraction, and possibly multiple extraction cycles. The final extract may be used directly or dried on an excipient. Thus, the production process leads to the loss of components found in the original ingredient and introduces new components associated with the excipient or from intentional addition of other ingredients and/or components. Verification of the presence of a botanical ingredient in a commercial supplement is dependent on identifying chemical components from the ingredient in the supplement.

Correlation (point by point multiplication) of spectra or chromatograms is a robust approach to identifying common components and is particularly useful for flow injection mass spectrometry (FIMS). Spectral correlation reinforces the counts of common components and diminishes the counts for noise or when one component is missing. An accurate correlation spectrum requires the use of authentic ingredients and supplements that are known to represent a common preparation method. The correlation spectrum can then be used as a filter, or template, to compare the common components of botanical ingredients and their supplements. Multiplication of ingredients and supplements by the correlation spectrum provides an array that reveals their similarity. Based on one-class classification for soft independent modeling of class analogy (SIMCA), it can be determined whether the supplements fit or lie outside the model established for the ingredients.

Keywords: Chemometrics, Mass Spectrometry, Natural Products, Pattern Recognition
Application Code: Bioanalytical
Methodology Code: Mass Spectrometry
Analytical Methods and Reference Materials for Dietary Supplements

Characterizing and Establishing Authenticity of Botanical Products

Current quality control approaches of establishing identity specifications are not only required for compliance with good manufacturing practices (GMP), but for prevention of economically motivated adulteration. Botanical authenticity is classically achieved by examination of diagnostic macroscopic and microscopic features, these techniques remain useful for minimally processed biomass such as dried roots. However, modern ingredient supply chains are often far removed from classically identifiable material and traded as highly pulverized dried plant powders. There is continuing demand for high quality, authentic products in the marketplace, and the volume of this demand significantly outpaces research efforts to produce reliable analytical methods that verify botanical identity. Technologies for acquisition of plant phytochemical profiles have developed rapidly, and in combination with chemometric analyses, shows promise as a tool for qualitative determination of identity and purity with respect to defined adulteration in botanical materials. A standard metabolomics data set contains vast amounts of information and key factors in using the data effectively are experimental design, availability of reference materials, sample preparation and selection of statistical analyses performed. This talk will demonstrate practical experiences in the development of methods using phytochemical profiling for characterization and authentication of botanical materials as a GMP requirement. Botanical materials such as Echinacea, Panax, Hydrastis, and Ligusticum species based on NIR and NMR plant metabolite acquisitions will be used to illustrate the utility of multivariate models to characterize and authenticate botanical materials.

Keywords: Food Science, Metabolomics, Metabonomics, Method Development, Natural Products
Application Code: Quality/QA/QC
Methodology Code: Chemometrics
FDA requires Good Manufacturing Practices in the manufacture of Dietary Supplements to ensure the identity, purity and potency of active ingredients and to safeguard against contamination and adulteration. Dietary Supplements cover a vast array of active ingredients ranging from vitamins and minerals to phytochemicals, plant extracts, and concentrates. Ensuring accuracy and potency can be an analytical challenge due to the complexity of the analytes and starts with reliable methods and require accurate Certified Reference Materials.

Many dietary supplements are complex mixtures of natural products. Active ingredients such as fat soluble vitamins and many phytochemicals are often sensitive to oxygen. Development of accurate standards for these analytes requires control of process from certification through formulation and packaging to ensure stability and accuracy of the reference standard. Reference materials should be characterized for identity, purity, and potency by techniques that are specific and accurate for the analyte. Potency or content includes assessment of related substances and other residual content such as water, solvent and inorganic impurities. Certification should assess content and homogeneity of content from vial to vial. Packaging and handling of reference materials must ensure stability through shipping and shelf life. Critical design criteria in development of dietary supplement reference materials include handling, certification methods, accelerated and real time stability, packaging, and transport. Design examples of Dietary Supplement Certified Reference Materials developed in accordance with ISO Guide 34 and ISO 17025 will be presented.

Keywords: Food Safety, HPLC, Liquid Chromatography/Mass Spectroscopy, Reference Material
Application Code: Other
Methodology Code: Liquid Chromatography/Mass Spectrometry
The National Institute of Standards and Technology (NIST), in collaboration with the National Institutes of Health-Office of Dietary Supplements (NIH-ODS) has been working to develop tools to help the dietary supplement industry establish confidence in their analytical procedures. The majority of the effort has been the development of matrix based reference materials with certified and reference values for toxic and marker compounds. The matrices are selected to represent the “average” materials in the marketplace and often consist of a raw botanical material and an extract of the botanical material. In some cases, a challenge material e.g. chocolate flavored protein powder, or a mixed supplement tablet is included in the suite of materials. Laboratories may use these materials to establish the accuracy and precision of their methods during method development, as quality assurance tools, or to establish in-house reference material traceability to NIST.

In addition to the materials, NIST often develops analytical methods for the determination of specific analytes in the candidate materials and these methods are published in the literature including information about sample preparation/extraction studies, chromatographic separations, detection, and approaches to quantitation. Additional information, such as qualitative thin layer chromatography data or DNA sequences are provided for authentication information. A dietary supplement laboratory quality assurance program has also been developed as an offshoot of the reference material program, allowing the community to demonstrate measurement capabilities and to compare method dependent results.

Keywords: Method Development, Reference Material
Application Code: Quality/QA/QC
Methodology Code: Liquid Chromatography/Mass Spectrometry
Isotopic analysis is the new frontier in atomic spectrometry to improve optimized information for the topic studied. The recent developments of Multicollector mass spectrometry uses now routinely a plasma ionization technology that allows promoting all the hyphenation developed with ICP/MS. Therefore we have developed a series of hyphenation using rapid analyte sample introduction strategies using either laser ablation, gas chromatography or flow injection technologies. Here we are working well below the ppb with rapid (few second) transient signals. This imposes then to develop an array of software to process and correct for the transient signal. However, these techniques are getting mature and can be routinely operational and applied either to environmental applications as well as food research or lately to clinical research. If the gain of information is huge, the precision after the software corrections only show a degradation of the precision of a factor of 2 or 3 but at low ppt range then. Some hyphenation techniques can also accommodate for speciation information also at sub ppt levels. These major advances in atomic spectrometry open up now an all-new array of applications in water research, environmental sciences, food sciences and traceability. We will review these major advances and forecast their potentials and limitations. These new technologies using species specific and combined isotopic information call for a whole new array of detectors. When achieved, the combined resources with rejuvenate and open a whole new array of environmental research as well as in many other domains such as food, forensic, and clinical applications.

Keywords: Agricultural, Elemental Analysis, Environmental Analysis, Ultratrace Analysis

Application Code: Environmental

Methodology Code: Atomic Spectroscopy/Elemental Analysis
Frontiers in Atomic Spectrometry: Isotopic Signatures for Novel Environmental Assessments of Non Co

Copper Isotopic Composition as a Valuable Cancer Biomarker for Animals and Humans

Since around ten years an emerging field of interest is the use of MC-ICP-MS measurement for precise isotopic composition in biological samples to determine small isotopic variations of Cu, Ca or Fe and interpret these variations for hemochromatosis, different types of cancer or osteoporosis. Copper isotopic variations seem to have a strong interest as a cancer biomarker with papers from different groups and is one of the most promising element for real application in medicine. Here, we will show results for whole blood cancer samples obtained from a large scale databases with clinical data (> 150 samples) linked to dogs with lymphoma or mammary carcinoma showing how to diagnose and interpret the dCu variation as a valuable tool for veterinary help in a clinical context. This type of technic was also used in wildlife (> 100 samples of different types of mammals likes Lion, Tiger, Jaguar or Panther) with promising results and outcome.

In this presentation, we will also show results from a strong database of serum samples from liver cancer humans (> 350 samples) and the link between copper variations in the tumor, peri-tumor obtained from liver resection and serum. These variations will be correlated with key gene expression like GAPDH, EGLN1 or STEAP3 in-vivo and invitro. All of these studies suggests that using copper isotopic composition as a biomarker in medicine can be a valuable tool if it is use in controlled clinical context.

Keywords: ICP-MS
Application Code: Biomedical
Methodology Code: Mass Spectrometry
ICP-MS is a sensitive and robust technology for element and isotope analysis. In the last years applications with short, transient signals and with a large variety of potentially unknown analytes got broader interest: (a) single particle ICP-MS aims at measuring elemental content of individual (nano-) particles. Signals last for only a few hundred microseconds and the elemental content, e.g. of natural, particles or colloids can show large variety. (b) 2D and 3D laser ablation imaging of geo- and biological samples reveal information far beyond pure bulk analysis. Such applications require measurement of large parts of the elemental mass range in only a few or even sub-milliseconds but common mass analyzers coupled to ICP are restricted in required performance. Sequentially measuring quadrupole (QMS) or scanning sectorfield (SFMS) instruments loose most of the transient ion beam in the scanning mass filter process. Multiple collector (MCMS) instruments provide a restricted set of analyte masses only.

Time of flight (TOF) based MS technology provides quasi simultaneous measurement of entire mass spectra with high time resolution. Experimental results of the icpTOF instrument will be used to explain how new sample introduction systems for liquid samples open new ways for quantitative multi-element analyses of individual nanoparticles. And they show how simultaneous mass spectra acquisition helps to do LA imaging quantitative, faster and in much more detail.

Keywords: ICP-MS, Laser, Particle Size and Distribution, Time of Flight MS
Application Code: Environmental
Methodology Code: Atomic Spectroscopy/Elemental Analysis
Calcium (Ca) circulates in serum at rather constant concentration and passes largely the first stage of filtration in the kidneys with many other nutrients that the body should better keep. These nutrients, including Ca, are effectively removed from the primary urine in the tubuli of the kidneys so that only a small fraction of the Ca from glomerular filtration enters the secondary urine which goes into the bladder. As Ca transfer from the serum into urine is not complete - on the contrary, most Ca is reabsorbed, it is therefore expected that a change in Ca reabsorption efficiency (and thus kidney function) would induce a significant difference in Ca isotopic composition between serum and urine that correlates with the amount of Ca discharged in 24 urine collections. This leads to a hypothesis that the main control over the Ca isotopic composition of the urine is the efficiency by which Ca is reabsorbed in the kidneys.

In order to test this hypothesis, our study is conducted upon the MC-ICP-MS Ca isotope analyses of serum and urine samples from 20 volunteers. The volunteers are all on a standardized diet, and given a caffeine supplement. Serum and urine samples are taken before and after caffeine intake for comparison. Caffeine is a diuretic that reduces Ca reabsorption in the kidneys and should therefore change the difference in isotopic composition between serum and urine. In parallel, parameters of renal function and Ca metabolism are also measured. This work will serve as a starting point for the studies of body Ca homeostasis and methodology development for the measurements of tubular reabsorption.

Keywords: Biological Samples, Biomedical, ICP-MS, Mass Spectrometry
Application Code: Biomedical
Methodology Code: Mass Spectrometry
The coupling of the Liquid Sampling Atmospheric Pressure Glow Discharge (LS-APGD) with an Orbitrap Mass Analyzer: A Potential Paradigm Shift in Isotope Ratio Mass Spectrometry

Frontiers in Atomic Spectrometry: Isotopic Signatures for Novel Environmental Assessments of Non Co

The field of isotope ratio mass spectrometry (IRMS), as it applies to elemental species, has two key output parameters: precision and accuracy. To achieve high levels of both, the mass analyzers of choice have been magnetic sector-field instruments with multiple ion collectors following the magnetic dispersing element. The rationale here is the simultaneous detection of the relevant isotopes, which minimizes the noise associated with ion source fluctuations. The two most common ion sources are thermal ionization (TI) and the inductively-coupled plasma (ICP), each having their operational pluses and minuses. One of the limitations in multi-collector IRMS is the informational bandwidth. Specifically, instruments are equipped with a limited number of detector elements, limiting multi-element IR analyses. In order to address these limitations, our laboratories have coupled a liquid sampling-atmospheric pressure glow discharge (LS-APGD) microplasma ion source to an Orbitrap mass analyzer. Our initial results have achieved IR precisions of <0.16% for 235U/238U, which meets the International Target Values (ITV) set by the International Atomic Energy Agency for LEU and natural uranium isotopic measurements. The LS-APGD provides the opportunity to analyze aqueous solutions, particulates, and gases, but at powers far less than the ICP. As a result, the microplasma can be coupled to mass analyzers using interfaces common to “organic” MS. The coupling to the Orbitrap requires no modification of the normal interface. While never intended for use in IRMS, the Orbitrap provides extremely high mass resolution (R>70,000) in comparison to sector-field instruments. A more subtle, yet crucial, aspect is the fact that all ions are measured simultaneously (at least in the sampled ion packet), thus minimizing noise contributions to the precision. We describe here initial efforts in this unique coupling, with figures of merit specific to the case of uranium determinations. Key instrument and data processing parameters affecting the quality of the IR data will be presented, with paths forward towards better performance presented. It is believed that this unique combination of ion source and analyzer may well represent a paradigm shift in elemental IRMS.

Atomic Spectroscopy, Elemental Mass Spec, Isotope Ratio MS, Mass Spectrometry
Homeland Security/Forensics
Mass Spectrometry
The recent introduction of novel instrumentation for compound analysis and several sub-2[micro]m particle size stationary phases is revolutionizing the field of Supercritical Fluid Chromatography (SFC). Significant retention and selectivity variation recently attributed to silyl ether formation on the particle surface has been observed. Evaluation of the reproducibility, reliability, and "universal" application of a new generation of sub-2[micro]m stationary phases (Torus 2-picolyamine, 2-PIC) for the analysis of small molecules by Ultrahigh Performance SFC (UHPSFC) has been carried out. The application of the Torus 2-PIC particle for compound purification using preparative SFC-MS will be discussed.
Supercritical fluid extraction (SFE) is widely used as an analytical method for extracting small molecule components, such as natural products, from a wide variety of matrices. The main advantages of using SFE compared to traditional sample extraction techniques including liquid-liquid extraction, soxhlet extraction, and solid phase extraction, are simplified sample handling, improved sample stability, and higher extraction efficiency. Here we will demonstrate the application of online SFE/SFC for analysis of pharmaceutical and food samples. Utilizing a fully integrated SFE-SFC-MS system, solid and liquid samples were first extracted by SC-CO2 with the addition of co-solvent modifier and then immediately analyzed by SFC-MS. Different parameters were assessed: temperature of the vessel chamber, pressure of the extraction fluid, time of extraction and gradient, and co-solvent modifier concentration. Multiple examples will be presented.
Supercritical fluid chromatography (SFC) has been widely used for chiral separations and purifications in the pharmaceutical industry as a powerful and environmentally “greener” separation technology. However, traditional high-performance liquid chromatography (HPLC) and flash chromatography techniques still dominate the purification of various achiral intermediates and active pharmaceutical ingredients (APIs) in pharmaceutical drug discovery and development areas. In this presentation, successful examples of purifications by SFC using achiral stationary phases help to illustrate the capability, uniqueness, and efficiency of SFC for achiral preparative purifications. Further investigation of the use of preparative SFC for the purification of achiral mixtures will broaden the scope of this important technology in pharmaceutical discovery and development.
Distributed usage of analytical and preparative LC/MS instrumentation among medicinal chemists has been employed at GNF for over 15 years. Recently we incorporated supercritical fluid chromatography (SFC) into this mix. This talk will focus on maximizing the use and benefits of SFC instrumentation for a large number of users.
Chiral resolution of unprotected acids, amines, and amino acids can be a challenge due to their high molecular polarity, poor organic solvent solubility, and limited UV response. Polysaccharide chiral phases have been widely used for separations of pharmaceutical intermediates due to their column durability and sample load-ability. This presentation discusses polysaccharide stationary phases for enantiomer resolution of several acids, amines, and amino acids used as starting materials for synthesis of pharmaceutical intermediates. Baseline resolution is achieved for these samples using supercritical fluid chromatography (SFC) by forming ion-pair chromatography. The amines can be separated using sulphonic acid additives and the acids can be separated by using alkylamines additives to form charge-neutral lipophilic ion pairs to interact with polysaccharide-type CSPs. The presence of both acid and amine additives in mobile phase can also improve the separations on some unprotected amino acids. The detailed analytical method developments and detection methods are discussed in this presentation.

Keywords: HPLC Columns, Pharmaceutical, Solid Phase Extraction, Supercritical Fluid Chromatography
Application Code: Pharmaceutical
Methodology Code: Supercritical Fluid Chromatography
The chirality of a drug can potentially have a large impact on its biological activity, metabolism and toxicity. Obtaining optically pure compounds has become increasingly important in the discovery of therapeutic compounds since one enantiomer can have positive therapeutic properties while the other can display non-therapeutic or negative biological activity. Due to the often difficult and limiting nature of achiral synthesis, chiral chromatography is typically applied to access stereochemically pure compounds. The application of Supercritical Fluid Chromatography (SFC) to chiral separations has proven very effective in recent years due to its many advantages over chiral HPLC, such as shorter retention times, higher efficiencies per unit time, and the reduction of organic solvent waste. At AbbVie, the Analytical and Purification Sciences (APS) group has provided a chiral preparative SFC service since 2009, which has steadily grown to impact over 30 projects and over 200 samples per year. This talk will discuss strategies to meet the growing need and variability of chiral separations within Drug Discovery, such as developing a streamlined scale-up approach, applying structure similarity software to minimize method development, and focusing on unique features offered in preparative SFC to address a broader range of chiral separations in a high-throughput laboratory.

Disclosures:

EJ and PS are employees of AbbVie. The design, study conduct, and financial support for this research were provided by AbbVie. AbbVie participated in the interpretation of data, review, and approval of the publication.

Keywords: Chiral, Chromatography, Method Development, Supercritical Fluid Chromatography

Application Code: Drug Discovery

Methodology Code: Supercritical Fluid Chromatography
The analysis of petrochemical matrices is challenging but a better understanding is of great economic importance (e.g. for product quality control, process development or to solve problems like catalyst fouling and pipeline clogging) and scientific interest. The method of GCxGC-TOFMS has been evolved to a powerful approach to qualify and quantify middle distillates [1] and to resolve the isomeric composition. With a high temperature GCxGC method this approach is extendable to the analysis of heavy petrochemical fractions/crude oils (up to carbon numbers > 65). The implementation of fast multi-reflection, ultra-high resolution TOF-MS (mass resol. > 50,000) for GC opens up a new dimension of separation. The information from HRMS (e.g. Kendrick mass defect or van Krevelen plot) is complementary to the isomeric distribution information obtainable by the GCxGC separation. The HRMS and GCxGC-information spaces could be combined to generate chromatographically resolved mass defect plots. If very heavy petrochemical matrices, which are beyond the capability of high-temperature GC separations, shall be analyzed, a direct insertion probe (DIP) is applied to vaporize the material directly in the vacuum of the HRTOF ion source. With DIP temperatures up to 400°C can be applied which corresponds to extrapolated boiling points up to 700°C in the high vacuum of the source and crude oils, distillation residuals and SARA fractions are now accessible and have been analyzed. In addition to the highly resolved mass distribution here also the vaporization distribution information is accessible as a function of the DIP-temperature program. This compares well to the analysis of non-GC transferable petrochemical compounds by the hyphenation of thermal analysis to soft PI-TOFMS where the molecular weight distributions are registered as a function of the TA-temperature program [2].


**Keywords:** Gas Chromatography/Mass Spectrometry, Instrumentation, Petrochemical, Time of Flight MS

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

**Methodology Code:** Gas Chromatography/Mass Spectrometry
### Abstract Text

Standard reference materials (SRMs) are complex natural matrix samples certified at the National Institute of Standard and Technology for the evaluation and validation of analytical methods for the determination of polycyclic aromatic compounds (PACs) in complex mixtures. PACs comprise a complex class of condensed multi-ring benzenoid compounds originating from a wide variety of natural and anthropogenic sources. The parent homocyclic species, which contain only carbon and hydrogen, are the familiar polycyclic aromatic hydrocarbons (PAHs). In addition to PAHs, incomplete combustion of organic matter leads to numerous heterocyclic compounds containing at least one heteroatom such as polycyclic aromatic sulfur heterocycles (PASHs). In this study, an analytical method was developed for the separation and identification of PAHs and PASHs in combustion-related SRMs. Due to the complexity of the sample matrix, the analytical method described requires multiple cleanup steps. Solid-phase extraction on an aminopropyl (NH2) phase is used to remove the polar components. The processed sample is then fractionated using a normal-phase liquid chromatography procedure on an NH2 phase. PAHs and PASHs are determined in the collected fractions via reversed-phase liquid chromatography coupled to fluorescence detection and gas chromatography/mass spectrometry.

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### Keywords:
- Fuels\Energy\Petrochemical, Gas Chromatography/Mass Spectrometry
- Liquid Chromatography, PA

### Application Code:
- Fuels, Energy and Petrochemical

### Methodology Code:
- Gas Chromatography/Mass Spectrometry
Gas chromatography (GC) is the preferred technique to analyze refinery gas. ASTM D1946 and UOP 539 provide comprehensive instrument setup and performance guidelines on traditional GC, which typically analyze fixed gasses and C1 – C6+ hydrocarbons in 30 minutes. Micro GC instrumentation combines Microelectromechanical System (MEMS) technology with a modular GC platform to perform parallel analysis on refinery gas sample, significantly reducing analysis time to 3 minutes. Micro GC Fusion provides rapid temperature ramping through resistive column heating technology. Each GC module’s temperature profile is independently programmed; extending the application range on each column when compared to isothermal operation. Traditional Micro GC uses an Alumina column to elute C3-C5 olefins, and a polydimethylsiloxane (PDMS) column to elute n-hexane. Using a temperature profile on Micro GC Fusion, optimal separation occurs eluting C2’s through n-heptane on an Alumina column. Through similar optimization of the PDMS column, heavier hydrocarbons such as BTEX and C7-C10 will elute, extending the traditional analysis of refinery gas.

Keywords: Fuels\Energy\Petroleum, Gas Chromatography, Petroleum
Application Code: Fuels, Energy and Petroleum
Methodology Code: Gas Chromatography
A stand-alone, industrial mass spectrometer is fast, sensitive, and able to measure a wide range of gases from the ppm level up to 100%. This has led to use in several continuous, online gas analysis applications in oil refineries.

Several recent updates to EPA regulations involve gas analysis. Hydrogen sulfide, total sulfur and BTU content of the waste gas being sent to flare must be measured continuously. H2S spikes above 162 ppmv require reporting along with root cause analysis. In the flare gas application the mass spectrometer simultaneously measures sulfur and hydrocarbon compounds, and can be validated using safe-to-handle, ppm-level sulfur standards. Benzene fenceline monitoring is another application that has recently received regulatory updates, lowering the required actionable levels to 9.0 µg/m³ (0.2 ppb). Mass spectrometry is one of the few real-time analytical techniques sensitive enough to meet this requirement.

The efficient operation of numerous fuel gas burners and process units is based on balancing many factors, and the composition of fuel, feed and effluent gas streams can provide critical information to the refinery control system. Analytical flexibility allows a process mass spectrometer to measure the bulk constituents of manufacturing gas samples as well as low-level contaminants, such as sulfur and chlorine compounds. A full, speciated analysis is necessary for calculating specific gravity, and Wobbe Index, important fuel gas parameters that the mass spectrometer can report along with BTU.

Keywords: Environmental/Air, Mass Spectrometry, Petrochemical, Quadrupole MS
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Mass Spectrometry
An inverse micro-hollow glow discharge was developed and tested for analytic utility against coals and mineral sorbents. Optical emission spectroscopy identified elemental content by atomic spectral signatures and concentrations by the relative intensities of the corresponding electronic transitions. Analyses against a suite of reference coals validated the micro-plasma’s analytical utility by linearity of its optical emission intensity with elemental concentration. Capability for measuring inorganic sorbents’ composition is also demonstrated. Element specific calibration coefficients (C) were calculated for the major and minor elements of coal and sorbent (C, H, N, S, Ca, Mg, Si, Al, Na, Fe, K). Analytical results using the microplasma system compared favorably with those using standard analytical instruments, such as a CHN analyzer and ICP for mineral elements, but with attendant instrument advantages of size, weight and power, at far lower cost.

Keywords: Atomic Emission Spectroscopy, Coal, Elemental Analysis, Fuels\Energy\Petrochemical
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Atomic Spectroscopy/Elemental Analysis
The determination of carbohydrates in lignocellulosic biomass hydrolysates is a crucial step in biofuel production process, though development of robust analytical methods is still a challenge. High performance anion-exchange chromatography with pulsed amperometric detection (HPAE-PAD) can be used to determine carbohydrates in these samples. HPAE-PAD has been shown to deliver fast carbohydrate determinations in biomass hydrolysates using the Thermo Scientific™ Dionex™ CarboPac™ SA10-4μm column. Determination of carbohydrates in acid-hydrolyzed corn stover was shown in Thermo Scientific Application Note 1089 (AN1089). The method uses electrolytically generated hydroxide eluent and a 62 mil gasket in the electrochemical flow cell to resolve the eight common biomass sugars (xylose, sucrose, arabinose, galactose, glucose, mannose, fructose and cellobiose).

The current work updates the system used for the carbohydrate analysis in AN1089. The new system combines flexibility and ease-of-use with high sensitivity and selectivity. The method proposed here separates eight common carbohydrate sugars in less than eight minutes (Figure 1), which allows for shorter sample turnaround times and reduced eluent consumption. The method is linear from 0.005 to 2 g/L for all eight carbohydrates. Using this method, carbohydrates present in 10 different biofuel samples were quantified. The overall retention time and peak area RSDs were less than 1.26%. More over the method proposed here is robust and did not show significant change in retention time as well as peak area after 200 biofuel sample injections (Table 1).

In summary, the method proposed here is convenient, precise, and robust. It will enable improved reliability for biomass-to-biofuel efficiency calculations.

**Keywords:** Agricultural, Biofuels, Chromatography, Fuels\Energy\Petrochemical

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

**Methodology Code:** Liquid Chromatography
Fourier Transform Infrared (FT-IR) spectroscopy is one of the primary analytical techniques for monitoring the degradation of in-service lubricants, offering fast analysis times and automation for high sample throughput. The infrared spectrum contains a wealth of information about the formation of degradation products as the oil ages and the presence of contaminants. Several ASTM methods exist for the analysis of in-service lubricants based on FT-IR spectroscopy.

An area of increasing interest is the measurement of the acid number (TAN) and base number (TBN) of in-service oils. These parameters are considered to be key indicators of oil quality and are related to the accumulation of acid and the depletion of the base additive package in the oil. Current standard methods for the measurement of TAN and TBN utilize slow and expensive titrimetric methods that use hazardous reagents.

TAN and TBN can be determined using the same FT-IR measurement used for other oil quality parameters in a matter of seconds. Calibrations can be generated for a wide variety or for specific oil types and additive packages and incorporated into a fully automated system. The process for developing an optimum calibration for a variety of oil types will be shown.

Keywords: FTIR, Fuels\Energy\Petrochemical, Materials Characterization, Petrochemical
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Molecular Spectroscopy
The source and history of recycled thermoplastics can affect their processability and impact the quality of the end product. Differential Scanning Calorimetry is a widely used technique for the screening of thermoplastic recyclates. New software capabilities now enable the composition of the recyclates to be identified by comparing the measured DSC curves to a database library of known materials.

In this paper, the various pieces of recyclates from a batch of recycled polyurethane were separated by color. The samples of each color were tested with a DSC using identical conditions. The DSC curves from each sample were automatically analyzing and identifying the thermal effects (such as Tg, crystallization and melting). The actually composition of the differently colored samples were then identified using the database library. This technique provides a powerful quality control method of screening incoming recycled material.
Non-aqueous Redox Flow Batteries (NRFBs) are emerging devices for electric grid storage applications. A major challenge in redox flow batteries is enhancing the ionic conductivity across the membrane while preventing the crossover of the anolyte and catholyte.[superscript 1] Recently, we reported that using redox active polymers (RAPs) in combination with size exclusion membranes, in contrast to ion selective membranes, provides a viable alternative that addresses these challenges and enables NRFBs.[superscript 1]

The electron transfer kinetics of large polymer redox mediators with hundreds to thousands of redox active centers in solution are not well understood.[superscript 2-3[/superscript] In addition, the existence of a preceding chemical step that mediates the redox chemistry of RAPs particles in solution highlights the importance of 3-dimensional charge hopping on these polymer nanostructures.[superscript 2] In order to gain a better understanding of the electrochemical properties of polymer particles, we have evaluated their reactivity in solution and as well-ordered monolayers. Because of their discrete nature, polymer particles can be self-assembled into compact arrays on conductive substrates. Here, we report on the electrochemical evaluation of polymer monolayers through advanced analytical tools such as scanning electrochemical microscopy, spectroelectrochemistry and rotating disk electrode voltammetry. We report on the redox properties, and the mechanism of charge transfer of these films. Our studies provide the required morphological control and electroanalytical tools for understanding charge transfer and mobility effects that are important for the performance of RAPs for better performing energy storage flow devices.

Our laboratory at SUNY-Binghamton has discovered a new class of nanostructured, \(-\)-conjugated, polymers generally referred to as poly (amic) acid. Poly(pyromellitic dianhydride-p-phenylenediamine)-PPDD belongs to this family of \(-\)-conjugated, biodegradable polymers with exceptional reducing and stabilizing properties. They have free carboxyl and amide functionalities which confer them with cation-complexing properties. These, together with their considerable steric hindrance effect make them to be perfect as nanoparticle stabilizer. This talk focusses on the use of PPDD as a reducing & stabilizing agent, immobilization matrix, and directional template for the synthesis of anisotropic silver nanoparticles (AgNPs). Particle anisotropy offers unique features and functions that are difficult to obtain by simply size-tuning of the spherical nanoparticles. Anisotropy also enables the optical properties to become tunable throughout the near-infrared and infrared regions. The talk also provides physical insights into the mechanisms of directional templating of anisotropic nanoparticles based on diffusion limited aggregate model and coalescence growth mechanism.
Novel substituted colored poly(urea-urethanes) have been synthesized by reacting substituted benzothiazole derivatives, 3-amino phenol and hexamethylene diisocyanate. The synthesized colored poly(urea-urethanes) were characterized by elemental analysis, IR, thermogravimetry and were applied on polyester fabrics. These dyes were found to give a wide range of colored shades with very good depth and levelness on polyester fabric. The dyed fabric showed good fastness to light and very good to excellent fastness to washing.
The detection and quantification of blood cells provide critical information about a patient’s health status. For example, low red blood cell (RBC) count could suggest anemic conditions. A number of sophisticated instruments, such as hematology analyzers, have been developed to perform these measurements, but their use is typically limited to well-funded, clinical laboratories. Limited-resource settings, however, are often deprived of these capabilities.

Paper-based microfluidic devices have matured as an analytical platform that is capable of developing diagnostic assays that provide reliable clinical information while addressing the practical difficulties of limited-resource settings (e.g., cost and ease of manufacture). To date, the focus on the use of these tools has been limited to immunoassays, clinical chemistry, and electrochemistry. In comparison, assays that detect or measure the properties of cells are underdeveloped. While there have been a few examples of detecting cells using paper-based devices (e.g., an agglutination reaction for blood typing), the traditional function of paper or other porous media in these devices is to remove blood cells from samples so that the cells do not occlude pores and inhibit the flow of fluid. We investigated the use of a paper platform to allow, rather than impede, the flow of cells through paper in order to establish methods for cell assays in paper-based devices. As a proof-of-concept, we developed a paper-based tool for the determination of the hematocrit—the ratio of RBC volume to total volume of whole blood. We investigated the effects the physical properties of paper had on the device for promoting the transport of RBCs in paper. The ideal conditions for the fabrication of our device were determined based on our observation of a linear relationship between lateral distance RBCs traveled and hematocrit. Our work provides information necessary for overcoming the challenges of cell assays in paper-based devices.

**Keywords:** Bioanalytical, Lab-on-a-Chip/Microfluidics, Material Science

**Application Code:** Bioanalytical

**Methodology Code:** Microfluidics/Lab-on-a-Chip
In this work, highly luminescent carbon dots (CDs) were synthesized by the hydrothermal method at 170°C for 12 h using carrot juice as a carbon source. The prepared CDs exhibited bright blue fluorescence under UV light illumination at 365 nm. The CDs show fluorescence life time of ~5.3 ns at excitation wavelength of 370 nm. The synthesized fluorescent CDs were well dispersed in water and characterized by UV-visible, fluorescence, dynamic light scattering and Fourier transform infrared spectroscopic techniques. The controlled release of mytomycin from the mytomycin-CDs was realized at pH values of 5.2, 6.2 and 7.4, respectively. The results of the cytotoxicity and confocal laser scanning microscopic images indicate that the mytomycin-CDs were successfully uptaken by HeLa cells without apparent cytotoxicity. The synthesized CDs show great potential as drug vehicles with good biocompatibility, sustained release of mytomycin from CDs, indicating that the CDs can act as a promising drug delivery system for therapeutic delivery and/or bioimaging applications.

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

**Application Code:** Bioanalytical

**Methodology Code:** Fluorescence/Luminescence
Three-dimensional culture models are more representative of in vivo cellular phenotypes and responses than the monolayer cultures traditionally used in research laboratories. We have developed a culture system in which we can engineer tumor-like environments with specific oxygen and nutrient profiles. The experimental control afforded by these 3D cultures, which are modular in format and able to incorporate many cell types and extracellular matrices, allow us to ask specific questions about oxygen (redox) biology. We are particularly interested in quantifying the relationship between oxygen tension, cellular metabolism, and susceptibility to chemotherapeutics. In this talk, I will highlight our recent advances in constructing breast and colon tumor models for studying drug metabolism. In particular, how sub-populations of cells in low oxygen tensions evade chemotherapeutics.

Keywords: Bioanalytical, Biomedical, Biotechnology
Application Code: Bioanalytical
Methodology Code: Microfluidics/Lab-on-a-Chip
Fabrication and Characterization of Transmembrane Protein Functionalized-Phospholipid Nanoshell Microarrays

In the past decade, microarray techniques have proven highly versatile to perform quantitative and multiplexed analysis of a wide range of desirable targets, such as genes and proteins. Transmembrane proteins serve as transducers of the extracellular environment into intracellular signaling, playing critical roles in cell function. However, membrane proteins are difficult to study because of their amphiphilic nature and the associated requirements of a lipid membrane to maintain function. Phospholipid nanoshells (PN) are a convenient biomimetic platform that is increasingly used to quantify biophysical and biochemical processes related to cell function. In this study, we report a new approach to generate arrays of transmembrane proteins via reconstitution into PN and subsequent organized immobilization into an array format. A 250x250 \(\text{[micro]m}^2\) square pattern was formed on glass coverslips and subsequently modified with sulfonate derivatives to facilitate the covalent attachment of aminated PN, surrounded by poly-ethylene-glycol (PEG) as a passivating layer to minimize nonspecific adsorption. Successful fabrication of the microarray was confirmed by the immobilization of aminofluorescein and successful grafting of PN on the microarray was demonstrated by the conjugation of fluorophore functionalized PN. The reported study holds great promise for developing a high throughput multiplexed screening platform for studies of cellular transport and signaling.

Abstract Text

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Keywords: Bioanalytical, Lab-on-a-Chip/Microfluidics, Membrane, Sensors

Application Code: Bioanalytical

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

Scanning Microfluidic System for Chromatographic-Based Binding Assays with Near-Infrared Fluorescence Detection

Microfluidic devices are becoming popular as a means for miniaturizing liquid chromatographic systems for use in small and portable devices. This project examined the development of a microfluidic system and chromatography-based binding assays using near infrared (NIR) fluorescent labels to achieve low limits of detection in the nanomolar range. Surface enhanced fluorescence in the presence of silver nanoparticles was also explored to obtain limits of detection in the picomolar range. Detection of the NIR fluorescent labeled targets was performed by utilizing a NIR fluorescence microscope that was combined with a scanning microfluidic platform for on-column detection. The channels of the microfluidic devices contained a monolithic support that was made of glycidyl methacrylate-co-ethylene glycol dimethacrylate, onto which binding agents such as human serum albumin were immobilized and into which the silver nanoparticle could be incorporated. These components are now being integrated and tested for use in formats such as drug binding studies and a displacement assay for the detection of drugs and other solutes in environmental or biological samples.

Keywords: Biosensors, Drugs, Fluorescence, Lab-on-a-Chip/Microfluidics

Application Code: Bioanalytical

Methodology Code: Microfluidics/Lab-on-a-Chip
Immune responses are governed by protein signaling in complex tissue environments. In the lymph node, proteins called cytokines are secreted and transported to nearby cells to help promote immune responses; however their diffusion coefficients and binding rates are unknown because methods for diffusional analysis in lymph node tissue do not exist. In addition, tissue inflammation is hypothesized to affect diffusion and binding but the extent to which this occurs is unknown. Here, we used a microfluidic platform to deliver picogram quantities of fluorescently labelled cytokine to microscale regions within slices of murine lymph node. Diffusion was monitored by fluorescence microscopy. First, the method was validated by delivering 10-kDa and 40-kDa FITC-dextran to slices of agarose gel and comparing the resulting diffusion coefficients to those obtained using standard methods (FRAP and microinjection). Next, we quantified the diffusion of three cytokines: tumor necrosis factor alpha (TNF-[alpha]), interferon gamma (IFN-[gamma]), and interleukin-2 (IL-2), which are representative of two major structural classes, vary in multimerization, molecular weight, and bind to various elements of the extracellular matrix. Lastly, we quantified the extent to which inflammation altered diffusion and binding constants in lymph node tissue. This is the first method to directly measure cytokine transport in live tissue slices, and in the future it should promote a deeper understanding of cell signaling kinetics and the development of drugs to target these molecules.

Funding: Society for Analytical Chemists of Pittsburgh Starter Grant Award, AAI Careers in Immunology Fellowship

Keywords: Bioanalytical, Lab-on-a-Chip/Microfluidics, Microscopy
Application Code: Bioanalytical
Methodology Code: Microfluidics/Lab-on-a-Chip
We are developing pressure-actuated integrated microfluidic devices to help diagnose the risk of a pre-term birth (PTB). The novelty of our work lies in the integration of solid phase extraction (SPE) for sample enrichment, fluorescent labeling, and elution into an injector for microchip electrophoresis (CE) separation, on a single platform. We previously developed a CE module in a three-layer poly(dimethylsiloxane) (PDMS) system.[1] As a next step, we are developing an automated multichannel device, integrating SPE and CE modules, for on-chip enrichment, fluorescent labeling, elution to an injector, and CE separation of PTB biomarkers. SPE of PTB biomarkers was performed on a reversed-phase porous polymer monolith fabricated inside the thermoplastic layer, whereas hydrodynamic control operated through peristaltic pumps and pneumatic valves in the PDMS layer. We initially showed successful loading, enrichment, elution, and separation of pre-labeled as well as on-chip labeled ferritin and lactoferrin (PTB biomarkers) on a single channel integrated device.[2] Currently, studies are being performed to optimize conditions and automate analysis on multichannel integrated devices. Additionally, we are working on a device integrating immunoaffinity extraction and CE. Our eventual goal is to combine all three modules in a fully automated, integrated microfluidic device for pre-term birth diagnosis.

References:
2. S. Kumar, V. Sahore, C. I. Rogers, and A. T. Woolley, Analyst, 2016, 141, 1660-1668

Keywords: Bioanalytical, Lab-on-a-Chip/Microfluidics, Sample Preparation, Solid Phase Extraction

Application Code: Bioanalytical

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

Investigating Reactive Nitrogen Species Using Microchip Electrophoresis with Electrochemical Detection

When microglia, the immune cells in the brain, are activated, nitric oxide (NO) is produced to fight infection. NO may in turn react with superoxide to produce peroxynitrite (ONOO⁻), a highly reactive species capable of nitrating proteins and disrupting cell function. The accumulation of ONOO⁻ in the body has been implicated in a variety of disease states including neurodegeneration in Alzheimer’s disease. Microchip electrophoresis (ME) is a useful method for the analysis of these reactive nitrogen species (RNS) and related compounds because its fast separation times allow for the detection of species with short half-lives. Electrochemical (EC) detection is a direct detection method that enables the monitoring of RNS in real-time without the use of derivatization. While the overall goal of this project is to develop an ME-EC method for the analysis of nitrosative stress in microglia, first separation conditions must be optimized for RNS and the molecules they nitrate in cells. Initially, the synthesis and sample preparation of ONOO⁻ were optimized to produce stable samples with high concentrations of ONOO⁻ for reaction with other species susceptible to nitration and oxidation. These samples were evaluated using capillary electrophoresis with ultraviolet detection (CE-UV) because all sample components absorb at 214 nm and ONOO⁻ selectively absorbs at 302 nm. Additionally, CE-UV was used for synthesis optimization to enable the automated analysis of sample degradation into nitrate over an extended period of time. These ONOO⁻ samples were then reacted with tyrosine and glutathione, two common targets of ONOO⁻ action in the body, and the products were tracked using ME-EC. In the future, microglia will be stimulated with a variety of inflammatory agents and these species of interest will be separated and detected using the ME-EC system in order to better understand the role nitrosative stress plays in neurodegeneration.

Keywords: Biological Samples, Electrochemistry, Electrophoresis, Lab-on-a-Chip/Microfluidics

Application Code: Bioanalytical

Methodology Code: Microfluidics/Lab-on-a-Chip
Capillary Electrophoresis - New Technology

Flow-Gated Capillary Electrophoresis Coupled with Alternate Injections for Rapid Quantitation of Biological Samples

Electrophoresis (CE) in a capillary or microchip is a powerful separation technique in terms of separation efficiency, speed, and cost. Over the last three decades, instrumentation and technologies for CE have significantly developed and thousands of papers are published annually. However, the practical application of CE in industry is ironically limited. A major complaint about CE is its poor reproducibility. Here, we will present a flow-gated CE system coupled with alternate injections for rapid quantitation of biological samples, which aims to improve its analytical reproducibility. A micro-switch was integrated with the CE system to supply two flows: sample and its standard addition. By alternating the two samples to the injection flow gate, the sample and its standard additions were alternately injected and rapidly separated (< 1 minute). The standard additions served as external standards for individual analytes. By using the one-point standard addition method, quantitation of multiple analytes were achieved rapidly by comparing detection response of a specific analyte with its standard addition. This strategy was able to compensate for the drift of the detection system, including optical focusing and capillary conditions. Experimental results showed greatly improved reproducibility and accuracy. This presentation will cover the basic idea, two microfluidic configurations, and their application in determining amino acids in urine and blood plasma. This strategy has been validated to be practical to improve the robustness of the flow-gated CE system in quantitative analysis.

Keywords: Bioanalytical, Capillary Electrophoresis, Lab-on-a-Chip/Microfluidics, Separation Sciences

Application Code: High-Throughput Chemical Analysis

Methodology Code: Capillary Electrophoresis
Enzymes are biological catalysts that facilitate chemical reactions in body for normal activity of cells and other organs. Phospholipid nanogels enhance the stability and performance of the exoglycosidase enzymes and are used to pattern enzyme cartridges within a capillary. Capillary electrophoresis improves upon conventional methods of enzyme characterization by reducing the amount of enzyme from millilitres to nanolitres and incubation times needed for enzyme reactions from hours to minutes. Phospholipid nanogels are utilized to create a stationary enzyme plug for in-cartridge mixing of substrate in the enzyme plug, which overcomes the diffusional limitation of substrate molecules that result in incubation-dependent rates. This method is used to evaluate the performance of several enzymes for different substrates to establish the Michaelis Menten parameters for neuraminidase and $\beta$-galactosidase. It is feasible to utilize differences in enzyme activity to determine linkage composition. The length and concentration of the enzyme plug is adjusted to specifically cleave different linkages without using costly enzymes to cleave specific linkages.

**Keywords:** Capillary Electrophoresis, Enzyme Assays, Lipids, UV-VIS Absorbance/Luminescence

**Application Code:** Bioanalytical

**Methodology Code:** Capillary Electrophoresis
Lipid molecules and their derivatives have garnered interest in recent years for their potential as biomarkers for various neurological diseases and cancers. The combination of a liquid phase separation on-line with ESI-MS is an attractive analytical technique for detecting and quantifying complex mixtures of lipids. In this study, microfluidic electrophoresis chips are used to perform separations of phospholipids and isoprostanes prior to on-line ionization and detection via ESI-MS. Using organic solvents as buffers for the electrophoretic separation allows for the hydrophobic lipid molecules to be solvated and separated. Initial results indicate that phospholipids with different head groups can be separated with baseline resolution quickly on a small chip using N-methylformamide (NMF) as the main component of the separation medium. Microchips are made from borosilicate glass and exhibit the necessary chemical resistance to incorporate a wide range of solvents into the separation medium. They consist of a cross layout for discrete electrokinetic injections of picoliters of sample and a make-up flow channel downstream from the injection cross to supply sheath liquid and create a closed current path for the electrophoresis. The electrospray “tip” is fabricated with a precision dicing saw such that the separation channel exits at the diced chip’s corner. The current work focuses on the addition of organic solvents (methanol, acetonitrile, propanol, hexane, and others) to the separation buffer and sheath liquid to fine tune the separation and ionization characteristics of lipid mixtures for applications in biomarker detection and discovery.

Keywords: Clinical Chemistry, Lab-on-a-Chip/Microfluidics, Lipids, Mass Spectrometry
Application Code: Clinical/Toxicology
Methodology Code: Capillary Electrophoresis
Sample preconcentration for capillary electrophoresis (CE) can be done either before or after sample injection. Large volume sample stacking using an electroosmotic flow pump (LVSEP) is a widely used after-injection preconcentration technique removing the long sample matrix into the inlet side. However, due to the lack of an outlet vial, it was difficulty to apply LVSEP to CE with electrospray ionization mass spectrometry (ESI-MS). We solved this difficulty by placing a vial supplying a run buffer during the matrix removal and sample stacking process but using a conventional sheath liquid CE/ESI-MS interface.

Single drop microextraction (SDME) was in-line coupled with CE/MS to provide sample enrichment before injection. By hanging an acceptor drop (~100 nL) covered with a thin octanol layer at the separation capillary inlet using a temporary out vial of run buffer as above, SDME could be easily coupled with CE/MS. Basic drugs in human urine such as methamphetamine, amphetamine, phenethylamine, methoxyphenamine, and mephentermine were extracted from a basic sample solution to an acidic acceptor drop, obtaining 130~150-fold enrichments with 10-min SDME-CE/MS.

One issue in SDME is stably keeping the drop attached to the capillary. Recently we demonstrated headspace in-tube microextraction (HS-ITME) using the liquid inside the capillary as an acceptor. Chlorophenols in an aqueous sample solution were extracted into the acceptor plug, simply by placing the capillary filled with a basic run buffer in the HS. The whole process of HS-ITME-CE was carried out in a fully automated manner using a commercial CE instrument without any modification of the instrument. For coupling with CE-MS, ITME does not require an outlet vial to form a drop, contrary to SDME. HS-ITME-CE-MS of chlorophenols and short chaing fatty acids in water was done without any additional measure as easily as HS-ITME-CE.
Open Tubular Electrophoresis in glass tubes was an active field about fifty years ago. A few decades later it gave place to electrophoresis in flexible fused silica micro-tubes, which became known as Capillary Electrophoresis (CE). The range of 20 to 75 µm for the internal diameter has then become standard for a great number of applications. Consequently, a variety of CE modalities were developed over this close-to-ideal platform. For instance, Capillary Gel Electrophoresis, Capillary Isoelectric Focusing, CE in Polymer Solutions, CE with Chiral Selectors, and even MEKC, which is a hybrid offspring of CE and liquid chromatography. The later allows neutral components to be analyzed with high selectivity. More recently, Flow Counterbalanced CE was demonstrated to give very large plate number allowing the separation of organic compounds with different isotopes and even isotopomers. In the present work we show that the plate number can be increased even further by using some unexplored jet properties. For instance, it is shown that the TCE format has some interesting capabilities: i) the EOF is absolutely irrelevant and does not need to be controlled, ii) a large number of matrices can be used, iii) cations and anions with similar mobility in modulus can be simultaneously analyzed in BGE without complexing agents, iv) thermal peak broadening can be partially compensated by a Poiseuille counter flow, v) a mixture of analytes difficult to separate can be left running for many hours until the desired resolution is achieved. As a result, very large plate number are obtained.
In order to address the national backlog in processing sexual assault kits, a purified sample of male DNA must be selectively retrieved from a gynecological swab. Effective elution of the sample from the swab and efficient separation of intact sperm cells from epithelial cells and other debris allow for a successful polymerase chain reaction (PCR) amplification and short tandem repeat (STR) analysis of the perpetrator DNA. Capillary zone electrophoresis (CZE) is a promising avenue to perform the cell separation as it boasts three major advantages over present technologies: small sample size allowing for multiple analysis of limited available evidence, rapid separation time compared to standard methods and single cell detection and collection when interfaced with our automated fraction collector (FC). In this work, a buccal swab is used to generate epithelial cells, which are mixed with human sperm. The mixture is lightly agitated and eluted in buffer to release sample. The sample is directly electrokinetically or pressure injected into CZE where whole cells and lysed cellular matrices are separated by their unique charge and size ratios. Preliminary experiments utilize laser detection to determine the migration time of sperm cells. Results show that sperm migrate in a confined band in less than 30 minutes. Using optimized CZE-LIF parameters, factors are replicated on a CZE-FC instrument where the sample is collected into individual wells on a 96 well plate. Light microscopy is presently used to confirm the separation and collection of purified sperm cells. The isolated sample will then undergo PCR amplification and STR analysis for forensic identification.
The free fraction for drug in blood and biological activity of this drug are often dependent on binding by the drug with plasma proteins. 1-Acid glycoprotein (AGP) is a heavily glycosylated protein that is found in human serum and has 45% of its mass resulting from glycan groups. AGP has one major binding site for drugs and acts as an important transport protein for many neutral and basic pharmaceuticals. However, the heterogeneous glycosylation of this protein is believed to result in multiple glycoforms that may have different drug binding properties. In this study, high performance affinity columns containing the lectin concanavalin A was developed and used to separate AGP into fractions based on their bi-antennary glycan content. These fractions were then collected and trapped on an anti-AGP immunoextraction column to study the interactions of the isolated AGP glycoform fractions with various drugs by high performance affinity chromatography. The AGP fractions were also characterized in terms of their general glycoform patterns by using capillary electrophoresis. The lectin affinity columns could process at least 200 g of AGP per injection and good resolution was obtained between the non-retained and retained AGP fractions at a flow rate of 0.025 mL/min or less. The binding capacity of anti-AGP immunoextraction was determined by frontal analysis to be 17 µg, or 0.4 nmol AGP. The retention of the drugs disopyramide, warfarin, propranolol, imipramine and chlorpromazine were then measured and compared for the AGP fractions that were acquired from the lectin column.

Keywords: Bioanalytical, Capillary Electrophoresis, HPLC, Protein
Application Code: Bioanalytical
Methodology Code: Liquid Chromatography
Capillary Electrophoresis - New Technology

Modified Silica Nanoparticles for Molecular Recognition and Fluorescent Labeling

Silica nanoparticles have been increasingly used in analytical chemistry during the last several years due to their good biocompatibility, no swelling in aqueous and organic solvents, and easy modification with different functional groups, etc. Functionalized silica nanoparticles had been successfully used as pseudostationary phase for the enhanced separation of organic acids and bases, drugs and proteins using capillary electrochromatography. Some organic molecules can be covalently bound to silica nanoparticles that can be used for molecular recognition. One such application for example is the introduction of chiral molecules on the silica nanoparticles surface. Nanoparticles that have this chirality or other molecular properties can be used as pseudostationary phase in enantioseparation. Since the structures of amino acids can be easily modified, amino acids, peptides and their derivatives would be candidates to act as chiral precursors for the synthesis of modified silica nanoparticles based chiral pseudostationary phase. On the other hand, high fluorescence intensity and photostability can be achieved by incorporating several fluorophores into a single label or sensor entity such as silica nanoparticles. Silica nanoparticles containing copolymerized dyes can be made of different sizes and using almost any kind of fluorophores that have suitable reactive moiety for the formation of the conjugate silica nanoparticles precursor. During these studies several visible and NIR dyes were utilized for encapsulation into silica nanoparticles backbone. These dye doped silica nanoparticles can be utilized in latent fingermark detection and to monitor the enzymatic catalytic activity in some microorganisms.

Keywords: Bioanalytical, Capillary Electrophoresis, Chiral, Nanotechnology
Application Code: Nanotechnology
Methodology Code: Capillary Electrophoresis
Fragrances are complex combinations of natural and/or man-made substances that are added to many consumer products to give them a distinctive smell, impart a pleasant odour, to mask the inherent smell of some ingredients, but ultimately to enhance the experience of the user of the product.

Many people suffer from allergies, which are due to an abnormal reaction of the body to a previously encountered allergen.

In the current EU Cosmetic Regulations (1223/2009), there are ‘currently’ 26 fragrance ingredients, 24 volatile chemicals and 2 natural extracts (oak moss and tree moss), that are considered more likely to cause reactions in susceptible people. These 26 fragrance ingredients must be indicated in the list of ingredients of the final product, if the concentration exceeds 0.001% in leave-on products (10 mg/kg) (e.g. a moisturiser), or 0.01% (100 mg/kg) in rinse-off products (e.g. a shampoo). The 24 current regulated allergens contain compounds from different classes, (phenols, cyclic hydrocarbons, alcohols, carbonyl compounds, esters and lactones) with different polarities, similar structures, many are small molecules (ions with low m/z) and many are also isobaric, which results in nonspecific fragment. This presentation will consider how hyphenating Ultra Performance Convergence Chromatography (UPC\textsuperscript{2}) with MS detection can be used to achieve specificity, selectivity and sensitivity for the analysis of fragrance allergens in perfume and personal care products in a fast 7 minute run.

**Keywords:** Consumer Products, Flavor/Essential Oil, Mass Spectrometry, Supercritical Fluid Chromatography

**Application Code:** Consumer Products

**Methodology Code:** Supercritical Fluid Chromatography
A ternary reversed-phase method was developed and validated for the separation of non-ionic sunscreen compounds Oxybenzone, Octocrylene, Avobenzone, Octinoxate, Octisalate, and Homosalate. Separation was accomplished using a 15 cm x 4.6 mm, 3.5 μm C18 Agilent Eclipse XDB column with a reversed-phase ternary blend of Water, Acetonitrile, and Methanol. Compounds were quantitated using external quantitation approach with a UV absorbance of 315 nm and a flow rate of 1 mL/min. A ternary blend was proven necessary due to the co-elution of certain compounds when binary separations of either Acetonitrile/Water or Methanol/Water were used as the mobile phase solvents. An individual study for the solvent and temperature behavior for each analyte was studied, as well as the DryLab® software modeling was used to confirm optimum separation conditions and method robustness. The developed method was found simple, linear, precise, selective, and accurate for determination of the six sunscreen actives.

Acknowledgment: Special thanks to Dr. Imre-Molnar of the Molnar-Institute for applied chromatography for his generous donation of DryLab® Software.

Keywords: Method Development, Separation Sciences, Validation
Application Code: Consumer Products
Methodology Code: Liquid Chromatography
A specific and sensitive High Performance Liquid Chromatography method has been developed for the determination of nicotine enantiomers in electronic cigarette liquids using reversed-phase and chiral phase parameters. Separation of racemic nicotine was achieved using a Waters xTerra MS C8 column with a Methanol:Phosphate Buffer:Triethylamine (40:60:0.5) mobile phase and a detection wavelength set at 260nm. Chiral separation was achieved using a Daicel ChiralPak AD-H column with a Methanol:Ethanol:Triethylamine (90:10:0.1) mobile phase and a detection wavelength set at 260nm. Linearity was performed for both the racemic and chiral separation of nicotine over a range of 100 to 1000ppm. The correlation coefficients for both separations corresponded to 0.999. Accuracy testing was performed resulting in a percent recovery of 100.1% and a %RSD of 2.088 for the reversed phase separation and a percent recovery of 100.3% with a %RSD of 1.666 for the chiral separation. The precision for the reversed phase and chiral separations showed %RSD values of 1.748 and 1.682 respectively. Specificity of the reversed phase method was conducted by subjected a sample and placebo to forced degradation conditions for 24-hours. Samples of electronic cigarette liquids were obtained from multiple online venders and though donations from Northeastern Illinois University students. The results showed a significant discrepancy between the labeled nicotine concentration and the amount determined though separation, with many samples containing double their label concentrations. The chiral separation results showed variation in the amount of R- and S-nicotine between samples.

Keywords: Chiral Separations, Chromatography, HPLC
Application Code: Consumer Products
Methodology Code: Liquid Chromatography
In 2003, an EU Directive restricting the use of allergenic compounds in fragrances was released. The Directive named a total of 27 allergens, stating that they should be labeled if present at >100 ppm in ‘wash-off’ products (such as shower gels), or >10 ppm in ‘leave-on’ products (such as perfumes).

Compliance with this Directive therefore requires that these compounds are identified and quantified accurately - a considerable challenge due to the complex matrix and wide concentration ranges involved. In addition, it has been proposed to expand the list of monitored allergens to over 100 different compounds, making the process even more demanding.

To tackle this issue, the fragrance industry has adopted comprehensive two-dimensional GC coupled with time-of flight mass spectrometry (GCxGC-TOF MS). The enhanced separation capacity copes with the most complex of matrices, while the commercialization of simple, consumable-free flow modulation devices has made routine use more feasible.

This study focuses on the use of parallel detection GCxGC-TOF MS/FID for confident identification and robust quantitation in a single analysis. Furthermore, Tandem Ionisation is incorporated for the simultaneous acquisition of both hard and soft ionisation spectra, providing unique confidence in the identification of terpene isomers.
The current National Institute of Occupational Safety & Health (NIOSH) definition for an Immediately Dangerous to Life or Health (IDLH) condition, is one that poses a threat of exposure to airborne contaminants when that exposure is likely to cause death or immediate or delayed permanent adverse health effects or prevent escape from such an environment [NIOSH 2004]. There are nearly 400 hundred compounds listed by NIOSH as IDLH. Some of the most toxic chemicals are Br₂, Cl₂, acrolein, AsH₃, chloropicrin, methyl isocyanate... In 1990, EPA published a list of 189 air toxics which are hazardous air pollutants known to cause or suspected of causing cancer. There are more than 250 chemicals regulated by the Occupational Safety and Health Administration (OSHA) that have permissible exposure limits (PEL’s) between 1 ppb and 2 ppm.

There is a definite need for real time portable analyzers that can detect toxic chemicals with ppb or even ppt detection limits. A compact portable GC with a photoionization detector (PID) has low ppb detection limits for many of these compounds and with an efficient low power thermal desorber can detect ppt levels for many of these toxics. A battery operated GC could also be used to protect first responders in areas where there have been spills of hazardous chemicals. Presently, a gas chromatograph/ mass spectrometer (GC/MS) located in a trailer is used at a number of spill sites although it is not an ideal solution.

We will develop and describe the columns and conditions for a GC/PID for 12 toxic compounds on the OSHA toxic chemicals list or the NIOSH IDLH list.

Keywords: Environmental/Air, Gas Chromatography, Industrial Hygiene, Volatile Organic Compounds
Application Code: Environmental
Methodology Code: Gas Chromatography
Environmental Analysis for Air Quality and Atmospheric Conditions

Determination of Thermal Oxidizer Destruction and Removal Efficiency with an Innovative FTIR / GC-FTIR Analyzer

Thermal Oxidizers (TOs) are widely utilized by the chemical industry to reduce their Hazardous Air Pollutant (HAP) emissions. Destruction and Removal Efficiency (DRE) requirements for these TOs can be as high as 99.9% reduction or higher. The ability of FTIR or other analytical technologies to measure 10s of ppmv is well documented, but those same technologies struggle when 10s of ppbv must be measured to demonstrate the DRE requirement. This becomes more difficult in the presence of complex emission streams. Additionally, the inlet and outlet to the TO must be monitored simultaneously, each with drastically different analyte concentrations.

An innovative technology has been developed that couples an FTIR gas analyzer (capable of real-time simultaneous monitoring for many compounds from % to high ppbv) with a GC-FTIR that can measure 100s of compounds in the ppmv to sub-ppbv range in near-real-time. These two coupled analyzers now allow for the monitoring of nearly any process or environmental gas stream. By using an FTIR as the detector for both measurements, nine orders of dynamic range can be obtained while routine calibrations and full compound separation are not required. Along with this novel instrumental technology, state-of-the-art analytical software allows for the detection and quantification of analytes that are 10,000 times lower than a spectrally interfering compound. Data demonstrating the operation of the FTIR / GC-FTIR and its software on monitored DRE samples will be presented.

Keywords: Environmental Analysis, FTIR, Gas, GC
Application Code: Environmental
Methodology Code: Integrated Sensor Systems
Electronic waste (e-waste) is currently the fastest growing hazardous waste stream that continues to be a challenging concern for the global environment and public health. The average useful life of electronic products has continued to decline, and obsolete products are being stored or discarded with increasing frequency. E-waste is hazardous, complex and expensive to treat in an environmentally sound manner. As a result, new challenges related to the management of e-waste have become apparent.

Plasctics, which contain heavy metals and flame retardants, are burned in open piles and release deadly toxic emissions. Thermogravimetric analysis (TG) has been used to characterize and compare, composition, and thermal behavior of a wide range of samples. Non-isothermal thermogravimetric provides fast and simple comprehensive information on mass changes, characteristic temperature ranges for reactivity and combustion on a larger scale of solid fuels. Although, there are some studies on the use of TG for coal and biomass combustion, there are no published studies wherein those on e-waste. The objective so this study is to demonstrate the application of an analytical technique that integrates thermal analysis with gas chromatography and mass spectroscopy to identify and quantify toxic compounds emitted during the burning of electronics waste at different oxidizing conditions. The goal of this study is laboratory simulation of open burning practices that often accompany undeveloped e-waste recycling operations, and to provide information about exposure risks to these emissions.

Keywords: Characterization, GC-MS, Thermal Desorption
Application Code: Environmental
Methodology Code: Thermal Analysis
Ambient air is polluted by many Volatile Organic Compounds (VOCs) coming from anthropogenic and natural sources. VOCs from PAMS, TO14 and TO15 lists can be measured in many ranges of concentration, from ng/m³ up to mg/m³ depending on the location of the measurements. Due to the large number of molecules, the complete separation of all compounds is difficult to perform using chromatographic columns. Therefore, it is difficult to quantify precisely all compounds using a chromatograph equipped with a nonspecific detector. The goal of this study is to perform automatic and continuous identification and quantification of VOCs using a dual Thermal-Desorber Gas Chromatograph equipped with two Flame Ionization Detectors (FIDs) and one Mass Spectrometer (MS). The device is required to identify automatically coeluted compounds by MS technology adapted to industrial context. The coupling of two different TD-GC-FIDs to a Quadrupole MS allowed by an elaborated multiplexer system is the originality of the project: one TD-GC-FID for monitoring of light compounds and one for heavy compounds with specific analytical conditions for each system. A measurement campaign in petrochemical new site shows the concentration of about 100 compounds at different steps of the commissioning. Thanks to the continuous measurements every 30 minutes, variations of concentrations during specific hours can be monitored. Potentially coeluted compounds like terpenes or organochlorinated are identified and monitored at ppt level. This fully automatic system allows non-specialist operators to access expertise level results.
This study focuses on air quality analysis by detecting trace levels of volatile organic compounds (VOC) such as formaldehyde, benzene, toluene, xylene, asetaldehyde etc. These gases occur in urban and indoor air. It can outgas from vehicles, commodities, cleaning agents, printings, paints and wood panels, but also tobacco smoke contributes largely for example to the indoor BTX concentrations. VOCs can affect human health and cause discomfort, irritation of the eyes, nose, and throat, problems with nervous system and at elevated concentrations finally death. Trace level monitoring of these gases is important when analyzing the quality of ambient or indoor air.

The high sensitivity (below ppb [1]) in a wide dynamic range of the proposed system is achieved with a silicon MEMS cantilever sensor coupled with an optical readout system [2] and laser source, which is operating at the fundamental vibrational absorption wavelengths of the analyzed gases. High selectivity is achieved by measuring the infrared spectra of the sample gas utilizing widely tunable external cavity quantum cascade laser sources (EC-QCL). Ultimately, the combination of these technologies allows the development of a handheld size device, which opens a plethora of applications in the air quality measurement industry.


Keywords: Environmental/Air, Photoacoustic, Trace Analysis, Volatile Organic Compounds
Application Code: Environmental
Methodology Code: Molecular Spectroscopy
Air is an important matrix for people breath approximately 20,000 liters of air a day; therefore, measuring air to determine if toxic compounds are approaching or exceeding toxicological levels, is important in maintaining public health.

Automated Thermal Desorption (ATD) coupled with Gas Chromatography (GC) and Mass Spectrometry (MS) using EPA method TO-17 is the best technique for the analysis of VOCs and SVOCs in air. A wide boiling point range of compounds are retained and recovered.

New sorbent tube technology has enabled the possibility of VOCs and SVOCs bringing a two analysis test into one solution. This patented technology will be discussed, and comparisons of analyses on other sorbent media will be provided.

This presentation will demonstrate, from several site studies, that there are SVOCs in addition to VOCs present in air. This one-analysis solution is accurate and precise. It is more profitable. And since solvent use is minimal (only needed for standard preparation), it is a greener solution than the two-analysis approach.

The component focus will be from the hydrocarbon range nC3 to nC24 for soil gas samples, and nC4 to nC44 for the analysis of volatiles through the 16 regulated polynuclear aromatic hydrocarbons (PAHs). Superfund and soil vapor intrusion sites will be the focused sites.

Method parameters, sampling procedures, analytical parameters, reporting limits and comparison of the results from the site study using both the SVI and the XRO-444 tubes will be discussed.
The new environmental regulations for ship emissions monitoring in sulfur emissions control areas (SECAs) will generate a global need for new measurement tools in the near future. The new regulations force cargo ships to use fuel that contains notably lower levels of sulfur than previously. New regulations cost around $45 billion per year to the shipping industry and the shipping companies can save around $10000 per day per ship by using illegal higher sulfur level fuel. Emissions monitoring is required to detect the use of illegal fuel.

The sulfur concentration of the fuel used by a ship can be calculated by measuring the ratio of carbon dioxide (CO2) and sulfur dioxide (SO2) from the emissions. Current technologies that are used in this application are UV fluorescence spectroscopy for the detection of SO2 and cavity ringdown spectroscopy for the detection of CO2. Currently there is no standard method for the detection of the CO2/SO2 ratio and using two different methods increases the inaccuracy and unreliability in the measurement.

Gasera addresses the emissions monitoring need with laser-based photoacoustic detection\[1, 2[/sup]. A compact, field deployable, instrument combining quantum cascade laser (QCL) and diode laser is used to accurately measure small changes in background sulfur dioxide and carbon dioxide concentrations. High resolution laser spectroscopy at low pressures is needed as the SO2 lines are normally buried under stronger absorption lines of water. Sub-ppb level detection limits for the SO2 can be achieved with a combination of high power QCL and ultra-sensitive cantilever sensor, and the achieved performance is demonstrated with laboratory comparison against the respective reference methods, as well as with the first results from a feasibility field campaign.


Keywords: Environmental/Air, Fuels\Energy\Petrochemical, Photoacoustic, Spectroscopy
Application Code: Environmental
Methodology Code: Molecular Spectroscopy
In an effort to unravel the complexity encapsulating our climate and the atmosphere, Atomic Force Microscopy (AFM) is used to study the surface tension of individual submicrometer aerosol particle mimics. Understanding the role of sea spray aerosols on the climate and the atmosphere, in particular, is of high interest, due to the fact that most of the Earth is covered by the sea. However, the aerosol effects are still poorly understood and there is a large degree of uncertainty from empirical and theoretical models. Despite its general success, Köhler theoretical model assumes that the surface tension component in the equation is a constant. However, through direct surface tension measurements, we reaffirm that the surface tension is not only a function of relative humidity or concentration, but is also strongly dependent on the chemical composition and the tendency to be surface-active. Here, we have performed extensive surface tension studies of 0.5 – 1 µm individual particles in ambient conditions, which include both atmospherically-relevant and good fundamental models of the following: electrolyte salts, carboxylic acids, carbohydrates, and biological systems. By extending well beyond the solubility limit of the chemical moiety, our data show strong deviations from the predicted values of surface tension. Moreover, equilibrium contact angle measurements have been incorporated, in tandem with surface tension measurements, in an attempt to understand the overarching role of these aerosols in our climate.

This work was funded by National Science Foundation through the Center for Aerosol Impacts on Climate and the Environment under Grant no. CHE 1305427.

Keywords: Aerosols/Particulates, Atomic Force Microscopy (AFM), Environmental, Microscopy
Application Code: Environmental
Methodology Code: Microscopy
Carbohydrates are common components in foods and beverages. Many of these are important for verification of food quality or authenticity and to identify or control reactions in fermentations or similar processes. Using a list of more than forty mono- di- tri- and tetra- saccharides important for food and beverage monitoring, we developed an anion exchange functionalized 4[μ]m particle size column that provides a very high efficiency carbohydrate-separation phase. The optimized column format is 150mm in length (2- or 4-mm ID) and employs a hydrophobic, polymeric, micro-porous resin coated with very small anion exchange latex that is stable between pH 0 and 14. This pH-stable packing allows use of eluents conducive to anodic oxidation of carbohydrates at gold electrodes (see example figure below) and delivers improved throughput compared to longer columns. We evaluated carbohydrate selectivity under several different eluent conditions using samples with complex matrix components including milk, sake and honey. An important feature of the new phase is selectivity control using different eluent hydroxide concentrations. We prepared a selectivity chart (k' vs. [hydroxide] at nine different values between 8 and 80mM). This table will permit users to identify conditions optimal for resolution of many sample types based on their saccharide compositions.
The mild roasting of plant seeds (e.g. green coffee beans or nuts) generates rich aroma due to formation of a multitude of flavour compounds. Thus roasting represents a very important value-generating process for the food industry. In order to better understand the flavour generation processes, modern on-line analytical approaches are needed, allowing to follow the processes in real-time. The formation of flavours in roasting processes can be on-line monitored by photo ionisation mass spectrometry (PIMS). PIMS has been applied successfully from investigations on evolved roasting gases from the interior of individual coffee beans up to industrial process monitoring settings at large-scale roasters with a coffee roasting capacities of more than 1000 kg/h (Hertz-Schünemann et al., J. Mass Spectrom. 48, 2013, 1253ff).

A particularly well suited tool for rapid laboratory investigation of food roasting processes in a thermal analysis device (e.g. thermogravimetry, TG) coupled to a PIMS system. In order to further increase the selectivity of TA-PIMS a newly developed optically heating fast-cycling gas chromatograph (fast OHGC) was applied. The fast OHGC is placed between the TA-oven and the PIMS-system and allows a very fast GC-cycle time with heating up from 50 to 250 °C and cooling down again to 50 °C in less than 30 s. With this system e.g. the evolution of iso-meric and isobaric key-flavour compounds such as pyrazines, furan derivatives, phenols and carbonylic compounds from the mild roasting of different nuts (e.g. peanuts) or green coffee beans can be followed with a time resolution of the cycle time (i.e. down to 20-30 seconds). The new OHGC technology and TA-coupling as well as applications of the new systems to different food processes (roasting of coffee, several different nut species) are described. Finally the implications for industrial and academic food research are discussed.

Keywords: Food Science, Gas Chromatography/Mass Spectrometry, On-line, Process Monitoring
Application Code: Food Science
Methodology Code: Mass Spectrometry
The bark of borututu tree ([i]Cochlospermum angolensis[/i] Welw.) has been traditionally used in Africa to treat malaria and liver toxicity. The bark is available as a dietary supplement, in cut form or as encapsulated fine powder, with claims for antioxidant activity and weight loss. Due to scarce literature reports on its chemistry and pharmacology, we performed a preliminary phytochemical investigation of borututu bark to establish some analytical quality standards for crude and finished products. A reversed-phase HPLC method was developed for fingerprinting the total extract. Diphenylpicryl hydrazyl (DPPH) free-radical scavenging assay was utilized to evaluate antioxidant activity of total extract, fractions and isolated pure compounds of the bark. Activity-guided fractionation of the total extract lead to identification of gallic acid and protocatechuic acid as the main antioxidant phenolic constituents of borututu bark. The three pigments cochloxanthin, dihydrocochloxanthin and dimethyltaxifolin were also isolated and, although less active as antioxidants, they can serve as additional quality markers together with the two phenolic acids. All isolated compounds were identified by spectroscopic methods, mainly NMR and MS.
Honey is a complex mixture of sugars produced in nature by honeybees. Sugar composition of honey is mainly dependent on its floral source and differs in various honeys. Fructose and glucose are the major components and account for almost 85-95% of the honey’s carbohydrates. The remaining carbohydrates are a mixture of di-, tri-, and several larger oligo-saccharides. Minor honey sugars are useful for the determination of floral origin.

We developed a High Performance Anion Exchange chromatography with Pulsed Amperometric Detection (HPAE-PAD) method to measure and quantify the entire profile of mono-, di-, and trisaccharides in honey. In this method, separation of individual honey sugars was achieved on the recently introduced CarboPac PA210-4μm column, which provides fast, high-resolution separations for most mono- through tetra-saccharides. Carbohydrate detection was by PAD with a gold working electrode and therefore no sample derivatization was required. This CarboPac PA210-4μm column separated 15 honey sugars (See Figure) with minimum sample preparation and an overall cycle time of 25 min. Honey sugars in 12 commercial honey samples were characterized and quantified by the described method. PAD is sensitive thus allowed the determination of low concentration carbohydrates in honey, while at the same time detecting the high concentrations of the major components, glucose and fructose. The method showed good precision and accuracy with recovery % range of 80-120%. In addition, we demonstrated that HPAE-PAD profiling enabled detection of the addition of industrial sugar syrups (adulteration) to honey samples.

Keywords: Carbohydrates, Chromatography, Food Identification, Food Science
Application Code: Food Science
Methodology Code: Liquid Chromatography
Solid phase microextraction (SPME) is a technique that can be used for the analysis of a wide variety of analytes in many different sample matrices. It can be used for sampling analytes from headspace or by direct immersion. In the case of pesticides, many have low volatility, and thus must be sampled by immersion of the SPME fiber into the sample. Some food samples pose a challenge with this approach due to the presence of fats, sugars, pigments, and other macromolecules. These can stick to the fiber and reduce its usable life and/or be transferred to the GC, where they may interfere with chromatographic analysis. This presentation will describe the development of an SPME method for the GC/MS analysis of pesticide residues from a heavy background food sample – baby food. Specifically, the advantages of using an overcoated vs a standard SPME fiber will be discussed. Optimization of parameters in the SPME method such as pH, salt addition, sample dilution and extraction temperature will be described. Results will be presented showing the final SPME method applied to samples of pureed peas and prunes spiked with pesticides included on the list described as part of EU directive 2006/125/EC for baby food. Data showing method accuracy, reproducibility and ruggedness will be presented.
This work presents studies on food formulations with protein addition using passive microrheology. Passive microrheology studies the mobility and displacement of micron sized particles which results from Brownian motion. The motion of particles induces local deformations of the sample, which are directly related to its viscoelastic properties.

Our technique is based on Multi Speckle Diffusing Wave Spectroscopy (MS-DWS), which consists of Dynamic Light Scattering (DLS) extended to an opaque media. With a patented algorithm, the backscattered interfering light can be analysed in terms of Mean Square Displacement (MSD), which is directly related to the viscoelastic properties of a sample. Moreover, the optical method allows to study especially weak gels without any applied shear, which avoids perturbation of the sample.

Nowadays, proteins are widely used in food preparations in order to increase the nutritional value of foods. However, proteins behave particularly in combination with fats, (bio-)polymers and other proteins. Phase separation or synergistic effects are the consequence. This work shows how passive microrheology can be used to evaluate the influence of a protein preparation in comparison to a reference product. Viscoelastic properties and gel points of the samples are determined, which helps the formulator to have an acceptable new, but nutritionally valuable food preparation.
Practical Considerations when Transferring Methods to Sub 2 µm GFC Columns for Bioanalysis

In the modern age of chromatographic column advancement we have seen movement from the traditional 3 µm and 5 µm particles to sub 2 µm particles. This technological advancement has not only occurred in reverse phase and HILIC columns, but has also become available in gel filtration columns as well. Just as is the case with their reverse phase counterparts, moving to smaller particles increases column efficiency and chromatographic resolving power. The drawback, however, is that the pressure that is required to operate these columns increases substantially. It has been reported in the literature that these pressure increases could potentially increase the instances of agglomeration when one is dealing with large biomolecules. Another potential side effect of the pressure increase is the increase of frictional heating inside the column. When dealing with biological molecules, substantial temperature increases could lead to denaturing of the proteins. In this talk we will look at these potential issues with transferring methods from HPLC to UHPLC separation modes, and give some recommendations as to ways to avoid problems with agglomeration and temperature effects in order to realize the benefits of UHPLC separations of large biological molecules in GFC.

Keywords: Bioanalytical, Chromatography, HPLC Columns
Application Code: Bioanalytical
Methodology Code: Liquid Chromatography
We have developed a low thermal mass 1.0 mm ID precolumn that can be cooled, focusing injected solutes and reducing sample-induced dispersion in analytical scale separations with 2.1 mm ID columns. In this work we extend our “temperature-assisted solute focusing” (TASF) approach to reduce volume overload in 75-150 μm ID, capillary columns to the larger, more commonly used 2.1 mm ID format. TASF works by relying on the temperature dependence of solute retention; during the injection high power thermoelectric elements (TECs) cool the column inlet focusing the sample, then rapidly heat to release the focused sample band for separation on the analytical column. The TASF approach is effective in capillary scale columns. We designed a 20 x 1.0 mm ID stainless steel TASF precolumn packed with 5 μm C18 particles to be placed between the injection valve and a 2.1 mm ID reversed-phase separation column. Precolumn geometry was optimized using numerical heat transfer simulations (COMSOL) to minimize temperature transient-induced velocity inhomogeneity. Precolumn temperature was changed using eight 1.0 cm x 1.0 cm TECs capable of delivering up to a total of 90 W of heating/cooling power. Preliminary experiments to evaluate precolumn performance demonstrated its potential to reduce volume overload for 50 and 100 μl injections of test solutes. Focusing and separation temperatures were 5 and 80 °C. Temperature transients were fast, up to 1051±19 °C/min. Reproducibility was excellent. We have begun to apply TASF precolumn to reduce volume overload effects between dimensions in selective, non-comprehensive two-dimensional liquid chromatography.

Keywords: Chromatography, Instrumentation, Liquid Chromatography, Sample Introduction
Application Code: Bioanalytical
Methodology Code: Liquid Chromatography
Two-Stage Temperature-Assisted On-Column Solute Focusing: Enhancing Concentration Sensitivity in Capillary High Performance Liquid Chromatography

When studying solute concentrations near detection limits, the simplest solution to improve sensitivity is increasing sample volume. When fixed volumes are required, capillary scale columns are utilized to achieve sufficient quantitation by decreasing sample dilution. As sample volume becomes many times larger than the column fluid volume, a decrease in performance is observed due to volume overload. On-column focusing minimizes these effects by manipulating separation conditions which result in a compression of the solute band. Previously, we have advanced the concept of temperature assisted solute focusing (TASF) to address this volume overload problem. TASF relies on solute retention’s dependence on temperature to transiently increase retention during the injection by cooling the column inlet with high power thermoelectric elements (TECs).

In this work, we use two-stage TASF to focus the injection band twice, first on an initial TEC and then again on a second cooled TEC. We have found the addition of the second focusing segment to induce a multiplicative focusing effect, further improving concentration sensitivity.

We have applied this two-stage TASF approach to monitoring reductions in basal concentrations of serotonin by coupling capillary LC to microdialysis online. With resting serotonin levels maintained on the pM scale, it has previously not been possible to measure decreasing basal levels. Using two-stage TASF, we have solved the volume overload problem for the analysis of serotonin in vivo. We have successfully injected sample volumes up to X-times the column fluid volume, achieving detection limits of ~X mol.

Keywords: Capillary LC, HPLC, HPLC Columns, Neurochemistry
Application Code: Bioanalytical
Methodology Code: Liquid Chromatography
A method was developed for the entrapment of proteins on a monolithic hydrazide-activated support using oxidized glycogen as a capping agent. These supports were prepared and tested for use in high performance affinity chromatography (HPAC). In this approach, the binding agent (i.e., human serum albumin, HSA) was physically confined within the pores of a hydrazide-activated support by using mildly oxidized glycogen. Glycidyl methacrylate-co-ethylene glycol dimethacrylate (GMA-co-EDMA) monoliths were chosen for use in this work because of their ability to be made into a hydrazide-activated form and the ability to control their pore size by varying their porogen ratios or polymerization conditions. The GMA-co-EDMA monoliths were modified with oxalic dihydrazide and used for the entrapment of HSA. The binding activity of these supports for model solutes that can bind to HSA was then examined as the conditions used to make these monoliths were varied. This approach is a general one that could be used with other proteins and that should allow these binding agents to be placed into monoliths in a soluble and highly active form for analytical or preparative applications of HPAC.

**Keywords:** Bioanalytical, HPLC Columns, Immobilization, Pharmaceutical

**Application Code:** Bioanalytical

**Methodology Code:** Liquid Chromatography
We present on the construction of an instrument designed to improve the performance of capillary liquid chromatography through the use of active temperature control (ATC). ATC means to program column temperature in a time and space dependent way. Using a linear array of ten, independent, electronically controlled, high power thermoelectric elements (TECs) we have created a system capable of complex spatial temperature programs. Column temperature can be regulated precisely at specific locations along the column’s long axis over a temperature range of 75 °C, with a minimum of -10 °C and a maximum of 100 °C. This design offers many advantages. For example, the first two TECs can be cooled to 0 °C during the injection to enhance sensitivity while the remaining eight are held at 75 °C. Following temperature-assisted focusing the cold TECs were heated to 75 °C at a rate of >1000 °C/min. In a second application of ATC the column was subjected to a negative axial thermal gradient. In this mode all ten TECs were initially at 20 °C. After injection the first TEC was heated to 90 °C in 5 minutes. The temperature program for second TEC was delayed 30 s, the third 60 s, etc., for all ten TECs. Thus the tail of the solute band was always travelling at a velocity greater than the leading edge, mimicking the effects observed in solvent gradient elution. We report on the improvements in chromatographic performance with the ATC system using a series of small molecule pharmaceuticals.

Keywords: Capillary LC, Chromatography, HPLC, Temperature
Autism Spectrum Disorder (ASD) manifests as impairments of social, emotional, and language development. The dynamic secretion of oxytocin from magnocellular neurons of the hypothalamus is correlated with the development and expression of these characteristics. Endogenous opioid hormones, like β-endorphin, are known to regulate oxytocin secretions, but the nature and dynamics of that regulatory relationship are not fully understood. To elucidate the regulatory relationships between oxytocin and endogenous opioid peptides, these hormones must be measured specifically, rapidly, and at biologically relevant concentrations (high pM to low nM). Immunoassays exploit the specific binding of Antibodies to their target analytes to achieve peptide hormone measurements with high specificity. Capillary electrophoresis (CE) has been utilized to achieve high speed immunoassays, but these assays are largely performed in a competitive format. Direct immunoassays offer analytical advantages for measuring analytes at low concentrations, but these assays are difficult to achieve by CE due to challenges of electrophoretic resolution. To address this, we are developing a strong cation exchange (SCX) chromatography method for direct immunoassay separations. Mobile phase composition, pH and salt gradients, and mobile phase velocity have been optimized to maximize separation speed while maintaining analytical separations. To achieve rapid measurements, column equilibration time was minimized by developing new column equilibration protocols. Future work towards improving immunocomplex stability on the SCX column will also be discussed.

Keywords: Bioanalytical, Immunoassay, Ion Chromatography, Method Development
Application Code: Bioanalytical
Methodology Code: Liquid Chromatography
Pillar array columns with a perfectly ordered structure were developed to provide improved separation efficiency compared to conventional LC columns. In our previous study, low-dispersion (LD) turns were used to increase the separation channel length without loss of separation efficiency. However, the LD turns resulted in a large pressure drop because the channel width of the LD turns was one-fourth that of the straight channel. Hence, in this study, we designed pillar array columns with low dispersion and low pressure drop (LD-LPD) turns. The LD-LPD turns had a constant width and a density gradient of pillars, with higher-density pillar arrays on the inner side, such that the inner and outer speed of the sample flow could be controlled to minimize dispersion in the turns. The pressure drop over the pillar array columns with LD-LPD turns was actually 7 times lower than that over the pillar array columns with LD turns. This result clearly shows that the pillar array columns with LD-LPD turns is preferable for separation at high flow rates. Separation of fluorescent derivatives of amino acids was successfully achieved in 1 min. The LD-LPD turns displayed low dispersion even at high flow rates and low pressure drop, which should give the advantage of fast separation of mixtures of biological compounds.
Neuropeptides have a variety of functions in the brain ranging from cell to cell communication to immune response and neuroprotection. The activity of many of these peptides is regulated by ectopeptidases, membrane-bound enzymes with active sites facing the extracellular space. Hydrolysis products can then interact with other receptors, eliciting effects different from the parent peptide. However, difficulty measuring ectoenzyme activity in vivo limits the understanding of how changes in ectoenzyme activity alter peptide effects. The most common approach to this problem is the use of microdialysis. While this is effective for identifying hydrolysis products, the inability to control the time which the substrate peptide is exposed to the tissue limits quantitative understanding. We have developed a two-probe technique known as electroosmotic perfusion – microdialysis (EOP-MD) for quantifying peptidase activity in vivo. By modifying a microdialysis probe to allow current to flow from a source capillary to the probe, substrate peptide is electroosmotically perfused into the extracellular space and hydrolysis products are collected by microdialysis. Using this technique, the residence time of the substrate is precisely known, allowing quantitative rate measurements. Furthermore, sampling can occur outside of the immediate vicinity of the probe and collection is not diffusion-dependent. By combining this method with the sensitivity of online capillary liquid chromatography – mass spectrometry, we are able to study regional differences in neuroprotective mechanisms in vivo in the rat hippocampus.

Keywords: Bioanalytical, Capillary LC, Peptides, Sampling
Application Code: Neurochemistry
Methodology Code: Sampling and Sample Preparation
The assessment of water quality demands for multi-target compatible analytical methods. On the one hand, the influence of sewage on natural surface waters must be routinely monitored. On the other hand, estimation of removal efficiencies of pollutants, such as drug residues, is in the focus of industrial and public wastewater treatment. Immunoassays, such as ELISA, are often applied in this area, however, it is only possible to measure a single analyte in one measurement.

In contrast, array technology is capable for measuring multiple substances in parallel. Here we present a four-plex bead-based flow cytometric assay for measuring three drugs (carbamazepine, an antiepileptic drug, diclofenac, an antiphlogistic and caffeine, a psychoactive substance) and the bile acid isolithocholic acid, which is proposed to be used as a fecal marker.

In this suspension array core-shell particles, consisting of a polystyrene core and a silica shell, are used. They can be easily encoded with a fluorophore which is introduced to the core while the shell is functionalized with amino groups to which the analytes or their derivates are bound via NHS chemistry. They are the competitor binding sites for the antibodies.

For the determination of the pollutants, the beads are incubated with a mixture of the analyte-specific antibodies. The molecules on the surface of the beads and the free analyte in solution compete for the binding sites of the antibodies. Bead-bound antibodies can then be visualized via dye-labelled secondary antibodies. For read-out a flow cytometer is used to virtually separate the differently encoded beads. In order to obtain highly selective binding of the antibodies, we investigated different types of surface modifications to overcome unspecific binding, finding that a PEG-based surface is suitable to support our immunoassay format. The resulting multiplexing assay is appropriate to detect the marker substances in the low [micro]g/L range.

**Keywords:** Bioanalytical, Environmental/Water, Immunoassay, Modified Silica

**Application Code:** Environmental

**Methodology Code:** Sensors
Hyperspectral remote sensing is invaluable for environmental monitoring because the sunlight reflected from vegetation involves its chemical and structural properties. Recently, we have the benefit of frequent opportunities of aerial monitoring using low-cost autonomous unmanned aerial vehicles (UAVs). In this work, we aim at the development of a new hyperspectral imager that can be mounted on lightweight UAVs.

In the project of “Development of three dimensional mapping system of marine macrophyte beds using hyper- and multi-spectral remote sensing from air and seasurface” supported by CREST, Japan Science and Technology Agency, we developed a hyperspectral whiskbroom imager using low-cost ultra-compact spectrometers (Fig. 1, 2, Table I). On the image plane, an image in the focal plane is quantized into a set of pixels. The composite plane is scanned along the cross-track direction by a swing mirror. The scanned light from each row is sent to a separate ultra-compact spectrometer C12880MA (Hamamatsu Photonics, Japan). Digitized radiometric data, time and position data (GPS) are recorded in a data storage. The number of spectrometers was four (three for the along-track scanning, one for skylight radiation). The gross weight was 2973 g.

An aerial observation of coastal area in the habitat of [i]Gelidium[/i] species around Izu Oshima, Japan was carried out on 25 and 26 February 2016 (Table I). Positional information of sea truth of three classes, [i]Gelidium[/i] bed (67), Coralline algal bed (67) and Sand bed (42), were identified by underwater survey. Fig. 3 shows color images taken on 25 February 2016 and the classification map based on pattern matching using the cross correlation. The high overall classification accuracies, i.e., 0.7547 on 25 February and 0.7188 on 26 February, indicate that the developed system can provide hyperspectral data with sufficiently high quality to detect underwater objects on the shallow coastal bottom.
Molecularly Imprinted Polyvinylidene Difluoride (PVDF) Sensor for the Detection of Hydrophobic Parathion Methyl Pesticide Molecules Using Quartz Crystal Microbalance

Molecularly imprinted polymer (MIP) has been widely applied in the preparation of chemical and biological sensors. Previous technologies mainly utilize functionalized polymer films such as polyacrylics (polyacrylic acid, poly(methacrylic acid)), polydopamine and polythiophene, or inorganic silica or titania films, biological antibody-antigen-antibody methods, fluorescent methods to detect and quantify pollutants in water. These methods mainly involve hydrophilic materials and hydrogen bonding. These sensors have advantages of detecting hydrophilic analyte molecules while they have difficulties to detect more hydrophobic analyte molecules in water. Here we demonstrate a hydrophobic polyvinylidene difluoride (PVDF) polymer sensor for the detection of hydrophobic parathion methyl molecules – a simulant for chemical warfare/threat agents. The PVDF sensor was prepared by MIP method with pre-polymerized PVDF. The prepared PVDF sensor had a high selectivity towards parathion methyl over other pesticide molecules like parathion ethyl, diethyl phosphoramidate, dicrotophos, paraoxon ethyl, and secbumenton and achieved a LOD of 68.0 nM and LOQ of 226.8 nM.

Keywords: Environmental Analysis, Environmental/Water, Pesticides, Sensors
Application Code: Environmental
Methodology Code: Sensors
Since an unbalanced excess of reactive oxygen/nitrogen species causes certain diseases, determination of antioxidants against oxidative stress is important in food analysis. Nanosensors have attracted much attention in antioxidant activity (AOA) assessment because of increased sensitivity and selectivity. Optical sensors offer advantages such as low cost, flexibility, speed, miniaturization and in situ analysis. Here we summarize the design principles of spectroscopic (colorimetric and fluorometric) sensors and nanoprobes for characterization of food antioxidants, and important milestones covered by our laboratory. We converted our CUPRAC (cupric ion reducing antioxidant capacity) determination method into an optical sensor by immobilizing cupric-neocuproine on a perfluorosulfonate-based Nafion membrane, where the CUPRAC absorbance on the sensor was a measure of AOA. We recently designed an optical oxidant/antioxidant sensor using N,N-dimethyl-p-phenylene diamine (DMPD) as probe, where ROS produced colored DMPD-quinone cationic radicals electrostatically retained on a Nafion membrane. The initial color was a measure of oxidative status (OS) whereas its attenuation by antioxidants enabled AOA estimation. The surface plasmon resonance (SPR) absorption of gold or silver nanoparticles (Au- or Ag-NPs) as a result of enlargement of citrate-reduced seed particles by antioxidant addition enabled a linear response of AOA. Ceria, titania, and magnetite nanoparticles can also be used as colorimetric sensors for measuring both OS and AOA. We determined biothiols with Ellman reagent (DTNB) derivatized Au-NPs, giving additive results in mixtures. Other thiol-type antioxidants can be estimated by selective adsorption or displacement reactions onto/from nanoparticle surfaces. We determined nitrite by means of 4-aminothiophenol-modified Au-NPs and naphthylethylene diamine as coupling agent.

Keywords: Food Science, Nanotechnology, Sensors, Spectrophotometry
Application Code: Nanotechnology
Methodology Code: Sensors
There are various approaches to biosensor fabrication for the electrochemical detection of non-electroactive species. However, these vary widely and result in a wide range of sensitivities to targeted analytes. The work presented herein focuses on first generation biosensing and quantitatively compares three distinct approaches to immobilization glucose oxidase on carbon-fiber microelectrodes. The microbiosensors are then coupled with fast-scan cyclic voltammetry (FSCV) for monitoring real-time glucose dynamics. This is achieved by exploiting the voltammetry of enzymatically generated hydrogen peroxide serving as a reporter molecule. We have characterized and systematically compared physical adsorption, hydrogel entrapment, and electrospinning as approaches to enzyme immobilization for first generation glucose biosensors. Of these, the hydrogel entrapment strategy is the most robust and reliable approach for glucose oxidase immobilization on the carbon surface. Moreover, the microbiosensors fabricated in this way are stable over several hours and provide sufficient sensitivity for measuring physiological concentrations of glucose in the brain. This tool is useful for monitoring sub-second glucose fluctuations in-vivo and our data is broadly applicable to the development of other enzyme-modified sensing methodologies for various nonelectroactive neurochemicals such as glutamate and choline.
Surface Plasmon Resonance Sensor is well-known as a highly sensitive signal transducer. In order to achieve a high sensitivity, immunoreaction is frequently employed. Although direct immunoassay and sandwich immunoassay are useful for a detection of common biochemical materials such as protein, sugar, etc., it is hard to detect a small analyte. Thus, we have studied an indirect competitive inhibition immunoassay, and it was noticed that this immunoassay showed remarkably high sensitivity for small analyte detection. Some research groups attempted to clarify the mechanism of the high sensitivity, but it is still under discussion.

The sensitivity of immunosensor is determined by both the affinity constant of immunoreaction and the sensitivity of the instrument. SPR already has a high sensitivity in nanogram scale of mass change at the interface. Because the affinity constant of immunoreaction is fixed by the nature of antibody, it is believed that the sensitivity is not controllable. Therefore, we proposed the control method of the affinity constant of the immunoreaction by using the modification to antibody with nanoparticle. The concept is quite simple. The equilibrium constant, so-called affinity constant of immunosurface, is determined by molar concentration of reactants and product. When the mass of reactant is increased by the modification, the molar mass concentration can be decreased. As a result, the affinity constant is proportional to the mass of reactant. This concept is theoretically confirmed by the modified Langmuir adsorption equation of the surface immunoreaction. It was noticed that the modified Langmuir adsorption isotherm equation predicted the sensitivity of the indirect competitive inhibition immunoassay.

We demonstrated this concept by the detection of beta agonist, clenbuterol. Clenbuterol is problematic material in food safety. It is proposed that the strategy to the ppq-level detection of clenbuterol in food sample.
Tetrabutylammonium fluoride (TBAF) is a frequently used source for fluoride in sensor development research. When using some commercial sources of TBAF in high concentrations, a pale yellow visible impurity is present at high concentrations. This yellow impurity has been noted to occur, even by commercial suppliers. This presents a problem however for the development of fluoride sensors as this impurity can lead to false positive results. We have identified that the source of this impurity is due to small quantities of iodine. Our research which we will present on is focused on identification, removal, and spectroscopic influence of this impurity.

Keywords: Identification, Sensors, Spectrophotometry, UV-VIS Absorbance/Luminescence
Application Code: General Interest
Methodology Code: Sensors
Examination of Cannabis and Hemp Products for Heavy Metal Contamination

The cannabis industry has taken the scientific world by storm, flooding the market with new products. Recently, concerns have arisen about safety of this, unregulated market, resulting in many new labs testing for cannabinoid potency, pesticides, bacteria/mold and other potential contaminants. Sadly, a potentially significant group of contaminants has been largely ignored: toxic metals.

Recreational cannabis and hemp are part of the C. sativa species, with different cultivars resulting in unique cannabinoid profiles. Federally legal hemp products (hempseed oil, hemp extracts, CBD oil & extracts), those not containing the psychoactive THC, are widely available on the market today. Such products are also used as a base oil for the addition of cannabis & cannabinoid extracts (including medical & recreational cannabis products). However, due to a ban on it’s cultivation in the US, virtually all of the hemp used is imported from China, India, & Eastern Europe. Studies of other consumable commodities exported from these countries have reported widespread heavy metal contamination (i.e. spices, teas, grains etc.).

Cannabis plants (hemp & recreational varieties) are bio-accumulators of heavy metals. In the production of many of the above mentioned products, a large amount of plant material is processed to extract concentrates and oils, thereby increasing the risk of heavy metal contamination. The scope of this study was twofold; firstly, to analyze various legal hemp products currently on the market and, secondly, to use these as a model for methods development for testing of restricted products. Samples were digested using microwave digestion and analyzed by ICP-OES and ICP-MS to determine the elemental composition of these products and the concentration of potentially toxic heavy metals. A large number of the tested hemp products detected heavy metal contamination.

Keywords: Consumer Products, Food Contaminants, Food Safety, Mass Spectrometry

Application Code: Food Contaminants
Methodology Code: Mass Spectrometry
Laser Induced Breakdown Spectroscopy (LIBS) is a fast and robust technique for elemental analysis. Trace amounts of inclusions and impurities as well as composition of alloys is obtained in seconds without sample preparation. LIBS removes the top microns of the sample that can be used to study the depth profiles.

In this work we will show how LIBS can be used for elemental analysis of painted surfaces. Paint analysis is often performed following traffic accidents to determine the model of the car involved. For such analyses, characteristic features of paint samples such as layer structure and chemical composition have to be obtained. Figure 1 illustrates the depth profile of a painted surface. Characteristic spectra of each layer were obtained without cutting the sample, as the laser pulse itself was used for ablation.

We will also show how non-metallic inclusions formed during steel production can be identified. Finally, we will demonstrate how LIBS can be used to accurately determine carbon content in stainless steel.

LIBS can be used in many fields: material science, automotive, oil, and metallurgical industries. Archeologists and mineralogists will benefit from ability to determine soil origin, detect minerals and trace elements immediately in the field. LIBS is of service to law enforcement as many samples of trace evidence including firearm residue, soil, glass and pigments can be determined fast and reliably with this technique. LIBS is an ideal technique for forensic examiners because of its ability to quickly identify large number of samples using virtually no sample preparation.

Keywords: Atomic Emission Spectroscopy, Elemental Analysis, Forensic Chemistry, Material Science
Application Code: Material Science
Methodology Code: Atomic Spectroscopy/Elemental Analysis
### Abstract Text

Study of six rare earth elements [cerium (Ce), europium (Eu), gadolinium (Gd), neodymium (Nd), samarium (Sm), and yttrium (Y)] has been reported using univariate and multivariate analyses. Oxides of these elements in powder form with varying concentration were separately and combinedly mixed with Al2O3 to make binary and complex mixtures respectively. Laser induced breakdown spectroscopy (LIBS) spectra obtained from binary mixtures were used for univariate analysis and those obtained from complex mixtures were used for multivariate analysis. Calibration models were developed using Simple linear regression (SLR) and Partial least squares (PLS) techniques. The limits of detections (LOD's) for Ce, Eu, Gd, Nd, Sm, and Y were determined from the calibration curves.

### Keywords

Atomic Spectroscopy, Elemental Analysis, Material Science

### Application Code

Material Science

### Methodology Code

Atomic Spectroscopy/Elemental Analysis
Nondestructive method was developed utilizing scanning angle (SA) Raman spectroscopy for simultaneously measuring the refractive index and thickness of waveguide block copolymer films that have varying fractional compositions of polystyrene-block-poly(methyl methacrylate) mixed with poly(methyl methacrylate) homopolymer (PS-b-PMMA:PMMA). The thicknesses of the films range from 495 to 950-nm and the PS-rich areas were controlled by varying the volume of PMMA homopolymer in the solution. All films were solvent annealed in toluene for 60 minutes, and then measured by SA Raman spectroscopy and fluorescence microscopy. The refractive index for the polymer films used in sum square electric field (SSEF) calculations was determined from SA Raman amplitude ratios of polystyrene’s 1001 cm⁻¹ and the 812 cm⁻¹ poly(methyl methacrylate) peaks. SSEF models of the experimental Raman amplitudes versus the incident angle of light were used to determine the film thicknesses. There was a 5% difference between the thickness measured by the SA Raman spectroscopy method and the value determined by optical profilometry. This method will be useful for monitoring mixed polymer waveguide films and in situ label-free analyses of films used in energy capture and conversion devices.

Keywords: Material Science, Method Development, Polymers & Plastics, Raman Spectroscopy

Abstract Text

Abstract Title: Determining Physical and Optical Properties of Thin Mixed Block Copolymer Waveguide Films by Scanning Angle Raman Spectroscopy

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Non-destructive nano-scale imaging of surface and sub-surface characterization has been a challenging problem in the materials sector. Inspection of semiconductor interfaces for < 10 nm patterns is a critical need for the next generation of 3D IC fabrication. Inspection of under-layer features via non-contact techniques is important for both in-process and post-process phases of IC fabrication and nanometer inspection metrology is an integral part of the yield ramp and process monitoring in semiconductor manufacturing. We describe a technique of 3D imaging with layer-by-layer inspection capability in a non-destructive fashion and with resolution < 1 nm. Terahertz (T-ray) frequency scanning instrumentation provides the precision metrology required to measure critical, nanometer size surface and sub-surface features on contemporary state of the art semiconductor devices. Measurements are made with a non-contacting probe that can accurately measure and graphically display features of interest with a resolution < 1 nm to process control engineers. T-ray is a narrow band, 0.3-3.0 THz or 1.0-0.1 mm, lying between infrared and microwave frequencies and often called the sub-millimeter band. We generate high-power, wide-band T-rays using an organic dendrimer dipole excitation or “DDE” [1]. This development in terahertz generation enables a non-destructive testing of semiconductor interfaces on a layer by layer basis [2]. As the wavelength of T-rays is microns to millimeter, a reconstructive approach has been designed that exploits the penetration capabilities of T-rays for probing sub-surface features. By means of “gridding to inverse power equation” a reconstructive technique has been designed that allows ~ 1 nm image resolution. Details of the methodology will be discussed with practical examples.

**Abstract Text**

Non-destructive nano-scale imaging of surface and sub-surface characterization has been a challenging problem in the materials sector. Inspection of semiconductor interfaces for < 10 nm patterns is a critical need for the next generation of 3D IC fabrication. Inspection of under-layer features via non-contact techniques is important for both in-process and post-process phases of IC fabrication and nanometer inspection metrology is an integral part of the yield ramp and process monitoring in semiconductor manufacturing. We describe a technique of 3D imaging with layer-by-layer inspection capability in a non-destructive fashion and with resolution < 1 nm. Terahertz (T-ray) frequency scanning instrumentation provides the precision metrology required to measure critical, nanometer size surface and sub-surface features on contemporary state of the art semiconductor devices. Measurements are made with a non-contacting probe that can accurately measure and graphically display features of interest with a resolution < 1 nm to process control engineers. T-ray is a narrow band, 0.3-3.0 THz or 1.0-0.1 mm, lying between infrared and microwave frequencies and often called the sub-millimeter band. We generate high-power, wide-band T-rays using an organic dendrimer dipole excitation or “DDE” [1]. This development in terahertz generation enables a non-destructive testing of semiconductor interfaces on a layer by layer basis [2]. As the wavelength of T-rays is microns to millimeter, a reconstructive approach has been designed that exploits the penetration capabilities of T-rays for probing sub-surface features. By means of “gridding to inverse power equation” a reconstructive technique has been designed that allows ~ 1 nm image resolution. Details of the methodology will be discussed with practical examples.

**Keywords:** Imaging, Nanotechnology, Semiconductor, Sensors

**Application Code:** Material Science

**Methodology Code:** Molecular Spectroscopy
Spectroscopic Applications in Materials Science

Graphene Oxide-Nanocarrier for Systematic In-Vitro Delivery of Antitumor Agents and FRET Based Detection of Ion Induced Enzymatic Activity

Graphene oxide (GO) has proven to be an attractive material whose chemical and physical properties make it highly adaptable to the fields of environmental sensing, drug delivery, and biotechnology. We report here the study of graphene oxide PEGylated derivatives (PEG-GO) for delivery of antitumor agent camptothecin (CPT) into HeLa cells. Strong pi-pi interactions between CPT and PEG-GO scaffolds afford loading of the agent in aqueous solution. Covalently attaching polyethylene-glycol (PEG) to GO offers two benefits: (a) to prevent interruption of biological function and (b) to allow candid monitoring of the cell via fluorescence labeling. UV-Vis was used to determine CPT loading efficiency and fluorescence spectroscopy monitored the slow release of CPT in PBS solution, which explains decreased efficacy over time and limited cell recovery. To confirm GO-nanocarrier’s suitability for in-vitro studies, several methods were utilized such as zeta-potential measurement and dynamic light scattering for size. The efficacy of GO-PEG, GO-PEG-drug, and drug alone were compared using SRB viability assays. Fluorescence microscopy has been broadly used for characterization of nanocarrier delivery into HeLa cells. We studied the potential of GO-nanocarrier for assisted FRET based monitoring using a peptide conjugate. Excess build-up of Ca2+ in the cell is known to promote apoptotic cascades and activation of calpain, a cysteine protease. Fluorescence imaging results indicate that, in the presence of various ion channel agonists and apoptosis inducers, GO delivery of peptide probe is highly sensitive to activated calpain. Inhibition experiments are carried out to verify specificity of calpain to the peptide linker. Combining FRET based tracking of enzymatic events with a nano carrier delivery system has great potential for pharmaceutical studies and for environmental monitoring.

Keywords: Biosensors, Fluorescence, Material Science, Nanotechnology

Application Code: Material Science

Methodology Code: Sensors
### Abstract Text

Adsorption of polyelectrolytes in membrane pores is a simple method for introducing ion-exchange sites into a membrane. However, development of polyelectrolyte multilayers with affinity groups that capture multilayers of protein is more challenging. Aiming to increase protein accessibility to affinity binding sites in polyelectrolyte multilayers, we synthesized and adsorbed star-polyelectrolytes to create coatings with large pores. Derivatization of adsorbed star-poly(acrylic acid) with aminobutyl nitrilotriacetate Ni²⁺ complexes yields membranes that selectively bind up to 50 mg of polyhistidine-tagged protein per mL of membrane. This binding capacity is comparable to that of commercial beads. To simplify the introduction of NTA groups in polyelectrolyte films, we developed a convenient synthesis of NTA-containing polyelectrolytes. Subsequent layer-by-layer adsorption of these polymers in membrane pores gives porous nylon membranes that capture 46 mg of polyhistidine-tagged ubiquitin per mL. Due to the high affinity of NTA for metal ions, Ni²⁺ leaching in binding and rinsing buffers is minimal. Adsorption of both star polyelectrolytes and NTA-containing polymers in porous membranes creates metal- and protein-binding materials that could serve as disposable membranes for purification of polyhistidine-tagged proteins.

### Keywords:
- Adsorption
- Material Science
- Membrane
- Sample Preparation

### Application Code:
- Material Science

### Methodology Code:
- Sampling and Sample Preparation
Glass microballoon syntactic foams consisting of 60-70 vol% hollow glass microballoons and epoxy resin matrix have gained considerable attention in recent years due to their unique combination mechanical properties and low density, with applications in the naval and aerospace industries. The primary limitation of these materials is the volume fraction ceiling (0.74) and subsequent density limit (0.355 g/cm³). Utilizing thermoplastic microballoons, syntactic foams were produced with densities as low as 0.067 g/cm³. These properties were achieved by developing a method that achieves void-free epoxy/microballoon compositions comprising an unusually high volume fraction of microballoons (Vmb = 0.75 – 0.95). The resulting morphology features microballoons which, having expanded in a restricted volume, are deformed into irregular shapes that efficiently pack together and are encapsulated by a thin coating of epoxy. The compressive yield strength, tensile strength and initial modulus of these highly loaded syntactic foams exhibits a non-linear decrease with increasing microballoon volume fraction with the sharpest decline generally occurring in the region of Vmb = 0.8 – 0.9.
### Abstract Text

The key component of a chromatographic system is the separation column, which contains the stationary phase attached to a support material; the chromatographic processes takes place within the column. Silica is the most widely used support material to attach the stationary phase for use in HPLC. Most recently, new approaches to column technology make use of silicon-based devices to prepare chromatographic columns, which takes advantage of well-established lithographic methods. Stationary phase attachment to silicon support is not quite as straightforward as it is with silica. The typical approach is to oxidize the silicon surface to provide hydroxyl groups to subsequently perform silanization. Such an attachment suffers of the same shortcomings of traditional silica modification. A different tactic is the use of diazonium chemistry, which can provide spontaneous grafting of an organic moiety on the silicon surface. This approach gives a facile route to create a silicon-carbon linkage leading to a more hydrolytically stable network than those obtained via typical silane chemistry. We demonstrate this approach by incorporating p-toluidine to the surface of commercially available silicon particles via diazonium chemistry. The modified materials have been characterized spectroscopically, packed into columns, and tested. This presentation will provide the details of the silicon surface modification, characterization as well as the preliminary assessment of the silicon modified particles.

### Keywords
- Characterization
- HPLC Columns
- Material Science

### Application Code
- Material Science

### Methodology Code
- Liquid Chromatography
A popular experiment in many general chemistry or quantitative chemical analysis laboratories across the country involves the analysis of limestone, a sedimentary rock formed from mostly calcium carbonate. Unfortunately, the analysis of limestone is problematic for several reasons. Because it is a sedimentary rock, many commercial limestone samples contain significant impurities. This causes considerable problems during sample preparation steps including difficulty grinding rock samples into powders, slow and incomplete attack by acids during digestion, and substantial filtering requirements. These aforementioned challenges can introduce significant error into the experimental procedure and lengthen the time of the laboratory experiment. Roanoke College is a private four-year liberal arts college of 2100 students located in southwest Virginia near the foothills of the Blue Ridge Mountains. Here, we designed an experiment for an inorganic quantitative analysis laboratory course around the analysis of Iceland Spar, an inexpensive metamorphic rock composed of solely calcium carbonate. Unlike the analysis of limestone, students were able to successfully grind and digest triplicate samples of Iceland Spar in less than 45 minutes and filtering was not required, allowing for more flexibility within the laboratory period and time for more sophisticated analyses. After digestion, students analyzed their solutions on an atomic absorption spectrometer and calculated mass percent of calcium in their mineral samples. Specific procedural details, student data, and student comments will be presented. Overall, the analysis of Iceland Spar provides students and faculty a streamlined, flexible, and enjoyable alternative over the more traditional analysis of limestone.
Analytical Education

Monitoring Water Quality of Abandoned Mine Drainage from a Passive Treatment Facility in Southwestern Pennsylvania: Getting High School Students Involved in the Research

This paper will focus on continued efforts by our research group toward monitoring the quality of abandoned mine drainage (AMD) processed by a passive treatment facility located at the site of the old Marchand coal mine near Lowber, Pennsylvania. This study is in collaboration with the Sewickley Creek Watershed Association (SCWA), a Greensburg, PA-based natural resources conservation group. The goals of these investigations are: 1) assessment of selected analytes in AMD from the Lowber facility in the field and laboratory, and 2) lending assistance to the SCWA toward assessment of AMD and development of remediation strategies for use at potential sites throughout the Sewickley Creek Watershed. In support of our group’s ongoing studies of water quality at the Lowber facility, a student from a local high school has joined our efforts and begun her own investigations. During the 2015-2016 academic year at Pitt-Greensburg, she has contributed to our work on monitoring levels of alkalinity, acidity, sulfate, and iron in mine drainage from the inlet pond (the location at which AMD emerges from the mine). In the 2016-2017 academic year, the focus of her work will be on conducting a survey of dissolved oxygen, iron, calcium, and sulfate levels in all six settling ponds as well as the inlet pond, outlet, and wetlands section of the Lowber facility. The hypothesis is that lower dissolved oxygen concentrations correlate with higher iron levels in the AMD, and that calcium levels in AMD are suppressed by high (ca. 1000 mg/L) sulfate concentrations. Sample collection, preparation, and analytical methods will be presented and discussed, as will results of determinations performed so far and their significance in terms of the effectiveness of the passive treatment process for removal of iron. Also to be presented and discussed are the benefits to high school students of involvement in this type of research experience, and future plans for this study overall.

Keywords: Environmental Analysis, Environmental/Water, Spectrophotometry, Teaching/Education
Application Code: Environmental
Methodology Code: Education/Teaching
Bring your children to work day is a national event in the United States which often excludes children with parents in the sciences due to lab safety protocols. This paper details a safe experiment which was designed to create interest in the sciences for all ages, while also demonstrating the capabilities of a new benchtop Time of Flight GC/MS to effectively differentiate some very exotic flavor profiles in store purchased jelly beans.

Single jelly beans were placed into respective 20mL HS vials along with 5mL of HPLC grade H2O and 1g of NaCl. A 2 cm PDMS/Carboxen/DVB SPME fiber was used to extract aroma compounds from the headspace of each jelly bean and deliver them to the GC–MS for analysis. An Rxi-5SilMS column was used for the chromatographic separation. The TOFMS data were acquired from 30-500 m/z at 20 spectra/second.

The benchtop time of flight GC/MS data were used to differentiate several pairs of jelly beans that had identical appearances, but vastly different flavor profiles. Examples of these visually identical jelly bean pairs which were differentiated include the following: lime / lawn clippings, berry blue / toothpaste, buttered popcorn / rotten egg, chocolate pudding / canned dog food, peach / barf, caramel corn / moldy cheese, and tutti-fruity / stinky socks.

The children were able to predict the good vs bad flavored jelly beans based on the published sensory descriptors for the analytes which were identified by GC/MS in each of the jelly bean varieties.

The kids found the experiment exciting and were exposed to analytical chemistry in a fun and educational way.
Microcontrollers and 3D printers are versatile prototyping platforms that once were exclusive to big companies. With the rise of the “Do It Yourself” (DIY) community those tools are now accessible to the final consumer and are finding space not only among the DIY community but also on chemistry laboratories. Although localized electrochemical techniques such as Scanning Electrochemical Microscopy (SECM) have been described on the literature for almost 30 years, it has not been widely adopted as other electrochemical techniques, being restricted to a small community, due to its complicated instrumentation, lack of variety on commercial alternatives and high cost. Herein a low cost alternative for acquiring space-resolved electrochemical information that use a microcontroller based potentiostat and 3D printed parts is presented. A commercial Arduino Uno (Arduino, Italy) microcontroller board is used as a potentiostat, position controller for the z-stage (perpendicular to the sample) and data acquisition system. The z-stage was built using an 5 mm diameter threaded rod encased in a linear stage printed out of PLA on a FDM 3D printer. The threaded rod is attached, with a 3D printed coupler, to a stepper motor that is controlled by the microcontroller in order to move the z-stage up/down. The microcontroller runs a custom built program to control both the z-stage and the potentiostat. With a platinum microelectrode (UME) attached to the z-stage using a 3D printed holder, the built equipment was used to both characterize the UME through cyclic voltammetry and to determine the tip-substrate separation/current dependence over an insulator substrate (silicon wafer) on a potassium ferricyanide solution. The resulting approach curves demonstrate the capability of acquiring space-resolved information by the proposed system. Further improvements are being made to allow for electrochemical images to be acquired through array scans.

Financial support: CNPq.
### Analytical Education

Including Broader Impacts in Your NSF-CHE Proposals

At the National Science Foundation, all research proposals must address both the Intellectual Merit and Broader Impacts criteria. The area of broader impacts may include scientific broader impacts, educational innovations, novel and/or far-reaching plans for dissemination, outreach activities, and efforts to broaden participation, particularly efforts to engage women and members of traditionally underrepresented groups in the STEM disciplines. This poster will highlight ways to address this important review criterion, with special emphasis on proposals submitted to the Chemical Measurement and Imaging (CMI) Program in the Division of Chemistry. Aspects of Broader Impacts related to RUI (Research in Undergraduate Institutions) and CAREER submissions will also be addressed. NSF staff will be available to discuss these, as well as NSF mechanisms for broadening participation, such as the Alliance for Graduate Education and the Professoriate and the Louis Stokes Alliance for Minority Participation.

### Keywords
Chemical, Instrumentation, Method Development

### Application Code
Other

### Methodology Code
Chemical Methods

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**Date:** Wednesday, March 08, 2017  -  Morn

**Time:**

**Room:** Exposition Floor, Aisle 2500-2600

**Primary Author**  Lin He

National Science Foundation

**Co-Author(s)**  Kelsey D. Cook, Marsha Y. Hawkins, Michelle Bushey
This poster will provide an overview of the programs and funding opportunities at the National Science Foundation (NSF) of particular interest to analytical chemists. Aspects of the CAREER, RUI, Chemical Measurement and Imaging, and Major Research Instrumentation programs; and the Computational and Data-Enabled Science and Engineering, BRAIN, Optics and Photonics, INFEWS, and BigData initiatives will be highlighted. Outcomes of the recently released reports on midscale instrumentation workshops will also be noted. Please come to this poster to interact with NSF staff and discuss available grant mechanisms and funding opportunities.

Keywords: Chemical, Instrumentation, Method Development
Application Code: Other
Methodology Code: Chemical Methods
Understanding biomolecule localization in specific tissues is critical in deciphering biochemical pathways, as well as in identifying and discovering biomarkers. Such biomarkers may have contributions in diagnosing cancer and other diseases. Matrix-assisted laser desorption/ionization time-of-flight imaging mass spectrometry (MALDI-TOF IMS) has proven to be a sensitive and effective tool for imaging and tracking biomolecules in tissue samples. While MALDI IMS allows the user to detect multiple species of varying mass to charge ratios in parallel, more analytes can be detected if they are extracted from the tissue. This project incorporates C18 tissue blotting techniques, as well as cross-linking magnetic beads, to actively extract peptides from tissue samples to improve detection by MALDI-TOF imaging mass spectrometry. This extraction is accomplished by utilizing a heterobifunctional cross-linker to conjugate peptides from a tissue sample to functionalized magnetic beads. After cross-linked peptides are immobilized onto the beads, the beads are blotted onto a C18 film using a magnetic separator where they are washed of any residual unbound analyte. Beads are then removed from the peptides by reducing the disulfide bond between them, leaving only the peptides on the C18 film which can then be analyzed using MALDI-TOF MS. While this cross-linking chemistry has been verified on a solid substrate, further studies using mouse tissue are planned. Using an active extraction tissue-blotting method could substantially increase the number of species obtained from tissue without compromising their spatial organization.

Keywords: Imaging, Mass Spectrometry, Method Development, Peptides
Application Code: Bioanalytical
Methodology Code: Mass Spectrometry
Applications of Mass Spectrometry

Investigations into Radiative Ion-Ion Neutralization as a Gas-Phase Ion Transduction and Spectroscopy Mechanism.

While Faraday plate detection remains the detector of choice for fielded ion mobility spectrometers (IMS), alternative detection mechanisms exist that promise to enhance sensitivity and selectivity. In this NSF-funded work, radiative ion-ion neutralization (RIIN) is investigated towards atmospheric pressure single-ion counting and spectroscopy. The RIIN interaction is induced by introducing IMS separated ions to an electrospray ionization (ESI) needle of the opposite polarity at the end of the IMS cell. The neutralization of ions produces light that is detected as the analytical signal. Building on previous work with a photomultiplier tube (PMT) as the photon detector, a monochromator/CCD camera was coupled to the system that allows wavelength discrimination of the RIIN photons following suppression of the nitrogen emission lines prevalent around active ESI or corona needles. IMS-RIIN spectra and signal-to-noise ratio for common mobility standards was obtained in order to compare results with traditional ion detection methods. In addition, the spectra of each of these compounds was obtained in order to provide insight into the RIIN mechanism and additional analytical information beyond the mobility of the ions themselves. Previous work demonstrated difficulties with positive mode IMS due to the production of light from the negative corona detector needle, and this issue is addressed in this work through the suppression of the nitrogen emission. Overall, the RIIN mechanism provides an alternative to traditional Faraday plate detection and its continued develop enable a second dimension of information to be attained that is truly orthogonal to the mobility domain.

Keywords: Detector, Electrospray, Multichannel Spectrometry (CCD CID array), Ultratrace Analysis
Application Code: Homeland Security/Forensics
Methodology Code: Mass Spectrometry
Forensic evidentiary backlogs are indicative of the growing need for cost-effective, high-throughput instrumental methods. In this work, we investigate an emerging technology that shows high promise in rectifying this problem, portable mass spectrometric (MS) instrumentation, which could also expedite and enhance criminal investigations along with allowing on-site evidence screening. The combination of portable MS with ambient ionization provides an ease of use and rapidity comparable to colorimetric field test kits, but with the discriminating power of MS (particularly, MS/MS analysis). While forensic applications demonstrated on portable MS systems are intriguing, there is an obligation to ensure that analytical performance of such technology is line with currently-accepted methodologies due to the inherent legal implications of collected data. This address with report the analytical validation of a portable, ambient sampling MS system implemented as a means of screening forensic evidence. The FLIR Systems AI-MS 1.2 cylindrical ion trap MS was fitted with an array of ambient and traditional ionization methods, including desorption electrospray ionization (DESI), paper spray ionization (PSI), paper cone spray ionization (PCSI), and direct sampling atmospheric pressure chemical ionization (APCI), and a systematic validation was conducted using forensic chemicals of interest (e.g. common drugs of abuse, cuttings agents, novel psychoactive substances (NPS)). Validation categories incorporated into this study include spectral accuracy, reliability (in terms of true detection rate and false negative error rates for large datasets), variability (in terms of inter/intra-day and inter/intra-user), sample throughput, limits of detection, method robustness, and environmental ruggedness.

**Keywords:** Drugs, Forensics, Mass Spectrometry, Validation

**Application Code:** Homeland Security/Forensics

**Methodology Code:** Mass Spectrometry
Solid-phase extraction (SPE) can easily and effectively enhance the sensitivity and separability in analysis. In contrast, in the use of this, the problems arose from the facts that were caused attenuating the recovery rate by sample ingredient or their matrix. Typically the recovery rates were calculated using the spike and recovery method and the certified reference materials. In all methods, measurement of recovery rate was separately conducted. In this study, a system which can simultaneously measure samples and recovery rate in just one-shot sample by online SPE-ICP-MS was presented. Switching valve on flow injection system, automatized measurement was conducted. Split-flow line was placed the main flow line prior the column, and the sample was divided by two-way using the splitter. One-way flows into column for preconcentration, and the other way flows into ICP-MS after dilution. After whole injection, all flow lines were washed using remover solution, at the same time of valve switching, target element is eluted / measured.

Column recovery rate was calculated measuring intensity / concentration of before and after solution through column. High accuracy measurement is resulted by the resultant recovery rate and leaded sample quantitative value. This method successfully applied to radioactive material analysis as strontium-90. In this case, the column recovery rate is measured using stable isotope in environmental. Although recovery rate fluctuation is derived by sample matrix, correct result leaded by appropriate correction. This method allows simultaneous measurement of quantitative value and recovery rate by just one-shot sample injection.

**Keywords:** Analysis, Flow Injection Analysis, ICP-MS, Nuclear Analytical Applications

**Application Code:** Nuclear

**Methodology Code:** Mass Spectrometry
Screening for Dioxin-Like Compounds in Sediment Using Modified QuEChERS and a GC-TOF Mass Spectrometer with Atmospheric Pressure Chemical Ionization

Comprehensive sediment monitoring programs require a large number of samples and present a large cost and workload for the analytical laboratory. The purpose of this study is to combine QuEChERS extraction with detection by qTOF-MS to decrease the cost and turnaround time for targeted monitoring of polychlorinated dioxins and furans as well as non-targeted identification of halogenated organics.

Samples from contaminated sites were extracted using a modified QuEChERS method, cleaned by carbon solid phase extraction, and analyzed using a Waters Xevo G2-XS QTOF (Wilmslow, UK) equipped with an atmospheric pressure gas chromatography (APGC) system (Wilmslow, UK) and a Zoex ZX2 thermal modulator (Zoex, Houston, USA) for comprehensive two-dimensional gas chromatography (GCxGC) experiments.

The modified QuEChERS method yields up to 30 samples/day compared to 10 samples in 3 or 4 days by the traditional Soxhlet-based extraction method. The proposed method produces higher MDLs and lower recoveries, but the measured toxic equivalence quotients differed by only 16±10% compared to traditional methodology.

The QTOF instrument used is capable of obtaining full-scan, high resolution (>20,000 FWHM) mass spectra in a time-frame compatible with GC and GCxGC experiments. The results show that the instrument performance is comparable to traditional magnetic sector instruments. It is also shown that GCxGC is capable of separating chlorinated DPE interferences that would otherwise necessitate a cleanup involving carbon SPE. Instrumental analysis using these approaches is compared to the classical method in terms of TEQs, precision, chromatographic separation, detection limits and workload capacity.

Keywords: Environmental Analysis, Environmental/Biological Samples, GC-MS, Mass Spectrometry
Application Code: Environmental
Methodology Code: Mass Spectrometry
Applications of Mass Spectrometry

VUV Lamp Based Chemical Ionization Ion Trap Mass Spectrometry for On-Situ Analysis of Drugs and Explosives

Mass spectrometry should solve two tough issues for on-situ analysis: the miniaturization and analytical time. Ion trap coupled with discontinuous atmospheric pressure interface realized the miniaturization. Ambient ionization realizes on-line analysis. In this study, a new chemical ionization source based on a vacuum ultraviolet lamp was designed and implemented in an on-situ ion trap mass spectrometry.

Methods:
A commercial 10.6 eV VUV krypton (Kr) discharge lamp with dopant gas was used for positive and negative chemical ionization source. Liquid and solid sample was collected on a thin film of PTFE, and a drift gas carry the gaseous samples generated from thermal desorption into the ionization chamber.

Results:
Acetone, butanone and water were chosen as the dopant gas. Twenty-seven different illegal drugs and ten explosives were texted, 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 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 humidity of gases promoted the detection of some compounds with higher ionization potentials that could not be detected well by single photon ionization, such as the detection of acids and alcohols. The results to confirm the on-situ performance, e.g. the stability and the sensitivity under under on-situ environment, will be presented.

Keywords: Chemical Ionization MS, Mass Spectrometry
Application Code: Homeland Security/Forensics
Methodology Code: Mass Spectrometry
Photon ionization (PI) is a powerful and attractive soft ionization method due to its high yield of molecular ions and very low degree of fragmentation. The coupling of PI and mass spectrometry (MS) has been widely used for online and in situ analysis of volatile organic compounds (VOCs) in complex mixtures. However, the applications of vacuum ultraviolet (VUV) lamp based PI-MS are limited due to the relatively low sensitivity. Herein, a novel high-pressure photon ionization (HPPI) ion source based on a 10.6 eV VUV lamp for a home-made time-of-flight mass spectrometer (TOFMS) will be presented. In order to achieve high ionization efficiency and sensitivity, the ion source pressure was elevated to about 700 Pa and the photoionization length was extended. PI-induced Chemical ionization (CI) was also realized through ion-molecule reaction with O2+ reactant ions generated by photoelectron ionization for the ionization and detection of analytes with high ionization potentials (IPs). As a result, limits of detection (LODs) down to mid-pptv levels for aliphatic and aromatic hydrocarbons were achieved. Furthermore, in-source collision dissociation (CID) was conducted for the acquisition of fragmentation information of analytes by varying the voltage between the ion source and the ion guide. Some isomeric VOCs can be easily distinguished according to their characteristic fragment ions without any additional separation technique. The HPPI-TOFMS was applied in a pilot study for online analysis of exhaled breath gas of humans. Our results suggest that HPPI-TOFMS can be a versatile and promising tool for online complex gas mixture analysis.
Assessing the extent of release of both engineered nano-materials and associated capping agents (surfactants) from nanocomposite packaging materials into foods is a necessary part of evaluating their safety. Such exposure assessments rely on the availability of accurate methods to determine the amount of additives in polymers. Here we report on the development of a method to quickly and accurately measure the concentration and distribution of organic surfactants within polymer films using Direct Analysis in Real Time (DART) as an introduction device for mass spectrometry. We compounded low density polyethylene (LDPE) films with surfactant-coated montmorillonite clays (Cloisite 20) as well as films with surfactants only. The surfactant we used was Arquad 2HT-75, a quaternary ammonium compound derived from hydrogenated beef tallow. The DART system vaporized small masses of the test films containing either Arquad 2HT-75 or Arquad-modified clays (Cloisite 20) for direct injection of liberated ions into an Exactive high resolution mass spectrometer. The four quaternary ammonium compounds from the Arquad surfactant were detected as the M-14 ions (loss of CH2): (m/z = 536.6 ), (m/z = 508.6 ), (m/z = 450.6 ), (m/z = 452.5). A lock mass (338.34058 m/z) ion was found in neat LDPE to maintain mass accuracy and account for drift that may be due to contamination of the instrument from the LDPE vapor. Relative standard deviations of replicate sample surfactant responses were reduced to less than 10% with optimized conditions. Linearity of response was evaluated for LDPE containing 0.5 to 4.0% surfactant; coefficient of determination (R2) values over 0.9 were obtained, indicating that DART-MS could be a quantitative method for surfactants or possibly other organic additives in plastic films.

**Keywords:** Mass Spectrometry, Nanotechnology, Polymers & Plastics, Surfactants

**Application Code:** Polymers and Plastics

**Methodology Code:** Mass Spectrometry
The benefits and consequences of pesticide use in the agriculture industry have been well reported in recent years with a concerted interest in biomonitoring populations for persistent exposure assessment. For this reason, efforts towards continuous improvement in analytical method development are standard practice in academic, government and industrial labs. In this study, a hot source induced desolvation (HSID) apparatus was employed to evaluate the potential of eliminating sample preparation in a pesticide analytical workflow for finished product fruit juices and wine samples. The anticipated impact would include higher throughput / lower cost method implementation in modern environmental surveillance /food safety laboratories. This work includes a repeatability study of no prep applications to demonstrate the robustness of this instrument platform. During the course of this investigation, observed limitations of the EU method for glyphosate analysis created an opportunity for further development towards reduced workflow and streamlined sample cleanup. Wine samples from crops sprayed with glyphosate during growth cycles and subsequent processing were evaluated.
Applications of Mass Spectrometry

Improved Instrument Robustness via a Hot Source Induced Desolvation (HSID) Interface for Tandem Mass Spectrometry Instrumentation

The emergence of liquid chromatography tandem mass spectrometry (LC-MS/MS) as a gold standard analytical platform for quantitative method development in high throughput toxicology, environmental surveillance and food safety laboratories has been well documented. Recent trends in practical considerations for improvements towards laboratory implementation focus on reduced downtime to facilitate testing methods for large sample populations. This poster will report on related strategies with a special focus on a dual spray ionization apparatus equipped with a heated coaxial flow ion source. The result yields a path for ion introduction into the orifice of the mass spectrometer using multi-orthogonal channels and laminar flow sampling. The advantages for this platform include high sensitivity due to an inherent reduction in chemical background (i.e. S/N, reduced N). Instrument ruggedness and stability are also improved due to orthogonal sampling and laminar flow. The laminar flow phenomenon is achieved by a combination of the influences of gas flow dynamics and electric fields. Ions are orthogonally extracted at atmospheric pressure and focused through a series of channels and turns entrained in a hot laminar flow of gas (different than traditional mass spectrometry instrumentation). Efficient desolvation is accomplished as a result of sequential energy transfer events. The flow of gas evolves through multiple transitions beginning with supersonic transitioning to shock cascading to turbulent and decreasing to laminar flow. The reduction in the speed of ion transmission is important to maintaining the sensitivity advantages of this interface.

Keywords: Clinical/Toxicology, Food Safety, Forensics, Mass Spectrometry

Application Code: Food Safety

Methodology Code: Mass Spectrometry
Over 30 toxin peptides and neuropeptides are known to undergo a unique post-translational modification of one L-amino acid near the N-terminus to a D-amino acid, forming a D-amino acid-containing peptide (DAACP). When compared to their all-L-amino acid counterparts, DAACPs have different 3D structure, bioactivity, and resistance to enzymatic degradation. However, both peptides have the same molecular weight, so this modification is difficult to observe by mass spectrometry (MS). We have developed an approach to identify DAACPs that takes advantage of their unique characteristics, with a goal of DAACP characterization that does not require standards for initial identification. We validate this approach using the neuropeptides from Aplysia californica, a mollusk where several DAACPs have been discovered. Suspected DAACPs are determined by their resistance to degradation by Aminopeptidase M (APM). This enzyme cleaves L- but not D-amino acid from the N-terminus. Digestion-resistant peptides are acid hydrolyzed into their component amino acids and their chirality determined via derivatization with 1-fluoro-2,4-dinitrophenyl-5-L-alanine amide followed by LC separation and detection with a triple-quadrupole MS. Once a D-amino acid is detected, final confirmation involves synthesizing the peptide with the D-amino acid and comparing its retention time to the endogenous peptide. Two peptides from a single prohormone, GYFD and SYADSKDEESNAALSDFA, resisted digestion by Aminopeptidase M, and both have D-tyrosine residues upon analysis of their amino acid content. They were confirmed to be DAACPs using synthetic standards with D-tyrosines in the second position from the N-terminus. Lastly, only GdYFD was found to be bioactive in the nervous system of Aplysia.

Keywords: Amino Acids, Chiral, Liquid Chromatography/Mass Spectroscopy, Peptides
Application Code: Neurochemistry
Methodology Code: Mass Spectrometry
The cerebellum facilitates movement and muscle memory by coordinating activities of several cell types (e.g. neurons and astrocytes). Traditional cell classification relies on immunostaining, which is not selective enough to detect the chemical heterogeneity found within previously defined cell types. Mass spectrometry (MS) detects hundreds of different compounds within a cell and has the potential to uncover chemical heterogeneity and reveal subpopulations within supposedly homogenous populations of cells. Unfortunately, cell fixation is incompatible with MS because the process removes or crosslinks distinguishing compounds. Here, we describe an MS-based classification of cellular subtypes by hyphenating immunostaining with high-throughput single cell MALDI MS, allowing correlation between the techniques.

First, cell displacement was evaluated for several MALDI matrix removal conditions to ensure that the cells would remain for subsequent immunostaining. In the optimized protocol, fluorescence microscopy of Hoechst 33342 provides cell locations for MALDI MS. After MS, the matrix is removed and cells are fixed simultaneously using a solution of paraformaldehyde in PBS. After anti-GFAP immunostaining, another set of fluorescence images are acquired to determine the location of cells and classify them based on their immunofluorescence. Because the cells do not move during this procedure, registering the initial fluorescence image with the post-immunostaining images links the single cell mass spectra with anti-GFAP expression. Therefore, mass spectra are extracted to determine the spectral profiles of thousands of individual astrocytes from the cerebellum. This procedure can easily accommodate additional antibodies and brain regions allowing for MS profiling of each major cell type in the brain.

**Abstract Text**

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

**Application Code:** Bioanalytical

**Methodology Code:** Mass Spectrometry
### Session Title
Applications of Mass Spectrometry

### Abstract Title
Consideration in Sample Preparation and ICP-MS Analysis of Biological Samples

### Primary Author
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### Co-Author(s)
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### Abstract Text
For many years the ICP-MS is a tool of choice for the trace analysis of elements like Pb, As, Hg, and Cu in body fluids such as urine, blood, serum and saliva. Single elements or panels of toxic or nutritional elements are run in the proper matrices providing doctors with comprehensive views on patient conditions. Recently, due to the popularity of implants, elements like Ti and Co were added to the common list of tested analytes. They are nontoxic, but can give medical providers information on an implant degradation. The goal of this poster is to demonstrate the capability of the current ICP-MS technology for trace element analysis in body fluids for research. A winning combination of reaction/collision spectral interference removal allows for the accurate determination of the low levels of analytes of interest.

It will be shown that the ICP-MS, in combination with an optimized sample introduction system, is a perfect technique for the analysis of diverse types of biological matrices including urine, serum and blood. One simple sample preparation technique, the appropriate diluent, and panels or individual analytes can be measured quickly and precisely. The results in reference materials will be shown and discussed.

### Keywords:
Atomic Spectroscopy, Bioanalytical, ICP-MS

### Application Code:
Clinical/Toxicology

### Methodology Code:
Mass Spectrometry
Applications of Mass Spectrometry

Determining the Dissociation Pathways of Gas-Phase Complexes Composed of Alkaline Earth Metals Coordinated by Alcohol Ligands

Electrospray ionization (ESI) is a proven method to generate gas-phase complexes composed of metal cations coordinated by a range of protic and aprotic solvent molecules. The gas-phase unimolecular and bimolecular reactions of the complexes can then be investigated using tandem mass spectrometry. The focus of this study is the formation and fragmentation of doubly-charged, alcohol-coordinated group II cations by ESI. The alcohol-coordinated alkaline cation gas-phase complexes, with general formula \([\text{M(ROH)}_n]^{2+}\) where \(\text{M} = \text{Mg}^{2+}, \text{Ca}^{2+}, \text{Sr}^{2+}\) and \(\text{Ba}^{2+}\) and \(\text{R} = \text{CH}_3, \text{CH}_2\text{CH}_3,\) and \(\text{CH}_2\text{CH}_2\text{CH}_3,\) were isolated in a 3-D quadrupole ion-trap mass spectrometer to determine the tendency for direct alcohol elimination or charge reduction reactions to create metal-hydroxide complexes. Direct alcohol elimination can occur as each alcohol ligand is removed from the metal complex until the complex can no longer undergo fragmentation. Charge reduction reactions can also occur in which as alcohol ligands are removed, the metal creates a metal-hydroxide complex, decreasing the charge on the complex to a single charge. The purpose of this experiment was to determine the extent to which (neutral) alcohol ligand elimination is observed for complexes with general formula \([\text{M(ROH)}_n]^{2+}\), and when charge reduction (whether by formation of metal hydroxide or metal alkoxides) becomes a competitive process.

**Abstract Text**
Electrospray ionization (ESI) is a proven method to generate gas-phase complexes composed of metal cations coordinated by a range of protic and aprotic solvent molecules. The gas-phase unimolecular and bimolecular reactions of the complexes can then be investigated using tandem mass spectrometry. The focus of this study is the formation and fragmentation of doubly-charged, alcohol-coordinated group II cations by ESI. The alcohol-coordinated alkaline cation gas-phase complexes, with general formula \([\text{M(ROH)}_n]^{2+}\) where \(\text{M} = \text{Mg}^{2+}, \text{Ca}^{2+}, \text{Sr}^{2+}\) and \(\text{Ba}^{2+}\) and \(\text{R} = \text{CH}_3, \text{CH}_2\text{CH}_3,\) and \(\text{CH}_2\text{CH}_2\text{CH}_3,\) were isolated in a 3-D quadrupole ion-trap mass spectrometer to determine the tendency for direct alcohol elimination or charge reduction reactions to create metal-hydroxide complexes. Direct alcohol elimination can occur as each alcohol ligand is removed from the metal complex until the complex can no longer undergo fragmentation. Charge reduction reactions can also occur in which as alcohol ligands are removed, the metal creates a metal-hydroxide complex, decreasing the charge on the complex to a single charge. The purpose of this experiment was to determine the extent to which (neutral) alcohol ligand elimination is observed for complexes with general formula \([\text{M(ROH)}_n]^{2+}\), and when charge reduction (whether by formation of metal hydroxide or metal alkoxides) becomes a competitive process.

**Keywords:** Bioanalytical, Environmental Analysis, ICP-MS, Quantitative
**Application Code:** Bioanalytical
**Methodology Code:** Mass Spectrometry
Coated Blade Spray (CBS) ionization was conceived as a fast and simple sampling/sample-preparation and ionization method for qualitative/quantitative mass spectrometry (MS) analysis of drugs of abuse, pharmaceuticals, and other small molecules from urine, blood, and other biofluids. This study describes the use of the CBS for the quantitation of immunosuppressive drugs from 100 µL of blood samples, focusing specifically on the simultaneous quantitation of everolimus, tacrolimus, sirolimus, and cyclosporine-A. These drugs are characterized for having a narrow therapeutic range (e.g. 2-15 ng mL-1, 5-15 ng mL-1, 6-8 ng mL-1, and 100-350 ng mL-1 for tacrolimus (TAC), sirolimus (SIR), everolimus (EVR) and cyclosporine A (CycA), respectively); therefore, continuous monitoring to avoid either sub-therapeutic or toxic effect is always required. Our results showed rewarding limits of quantitation (1 ng mL-1 for EVR/SIR/TAC, and 10 ng mL-1 for CycA) as well as good accuracy (≥90 %) and linearity (R2>0.99) over the range evaluated for all the compounds (1-50 ng/mL for EVR/SIR/TAC, and 10-500 ng mL-1 for CycA). The precision of the method was below 15% in all the cases and the bias for three different validation points ranged from 2% to 10%. No matrix effects were observed when evaluating this methodology at different hematocrit levels. By using a high-throughput autosampler, a total analysis time of less than 3 minutes can be attained per sample.
Applications of Mass Spectrometry

Targeted Screening for 75 PDE-5 Inhibitors and Analogs Using Three Different Analytical Approaches

There are currently four phosphodiesterase type-5 (PDE-5) inhibitors (sildenafil, tadalafil, vardenafil and avanafil) approved as active ingredients in drug products prescribed for treatment of erectile dysfunction (ED) in the United States. However, these innovator drugs along with unapproved analogs of these compounds have been observed in a variety of products available with or without a prescription. Screening for PDE-5 inhibitors and analogs in pharmaceutical dosage forms and dietary supplements marketed for treating ED is a commonly requested analysis at the Forensic Chemistry Center. Some manufacturers of “sexual enhancement” products regularly include new analogs in their formulations in an attempt to circumvent detection. In order to improve the screening method for PDE-5 inhibitors and analogs, the list of target compounds has been expanded to 75 entries, and we have adapted the method to include three possible instrumental approaches to maximize application in other laboratories. The screening method has been developed for use with traditional liquid chromatography with mass spectral detection (LC-MS), LC with high resolution accurate mass-mass spectrometry (LC-HRAM-MS), and direct analysis in real time with HRAM-MS (DART-HRAM-MS). These three techniques each have their own advantages, and adapting the method for use in each technique allows the analyst to apply the most appropriate strategy for efficient identifications. The expanded list of target compounds and the use of advanced technology to analyze these illicit products has also greatly facilitated identification of previously undiscovered analogs.

Keywords: Drugs, Liquid Chromatography/Mass Spectroscopy, Mass Spectrometry, Pharmaceutical

Application Code: Pharmaceutical

Methodology Code: Mass Spectrometry

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<td>Primary Author</td>
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New advances in technology are accommodating the need for higher sample throughput while maintaining or improving detection levels. This is especially true with respects to mass spectrometry. Mass spectrometry is predominantly the analytical tool of choice for most of the United States Food and Drug Administration’s (FDA) regulatory field laboratories. Traditionally, most mass spectrometers were coupled with some sort of device that introduced the sample (i.e. high performance liquid chromatography, gas chromatography, etc.) into the mass spectrometer. Although effective, these devices necessitate a considerable amount of time to provide acceptable results. Additionally, tedious and labor intensive sample preparation and extractions are often required for analysis using the aforementioned technologies. Incorporating the philosophy of continuous improvements toward enhancing public health protection, the FDA’s Arkansas Regional Laboratory has developed a rapid and an automated mass spectrometry screening method for twelve commonly found sulfonamides. Our research has demonstrated that an automated solid phase extraction system that is directly coupled to a mass spectrometer is an effective alternative to the traditional liquid-liquid extractions and LC/MS/MS analysis. This screening method can perform unattended and automated sample extraction and analysis of sulfa drugs at < 10 ppb for honey on a triple quadrupole mass spectrometer. Furthermore, each individual sample can be extracted and analyzed in less than 30 seconds which will enable us to accommodate a larger volume of samples and shorten sample turnaround times.

Keywords: Drugs, Food Safety, Mass Spectrometry, Sample Introduction
Application Code: Food Safety
Methodology Code: Mass Spectrometry
In order to meet the needs of the international nonproliferation and safeguards communities for a field deployable ionization source capable of highly accurate and precise isotope ratio (IR) measurements, the liquid sampling-atmospheric pressure glow discharge (LS-APGD) ion source is being developed. As part of the evaluation of IR qualities, the microplasma has been interfaced with Orbitrap mass analyzers. It has previously been shown that the LS-APGD is capable of producing a plasma with a higher energy density than found in ICP sources, all while requiring fewer consumables for operation making it an ideal candidate as a field deployable ion source. The LS-APGD /Orbitrap combination has been shown to measure a natural U sample ($^{235}\text{U}/^{238}\text{U} = 0.0072$), as 0.007003, with a relative standard deviation of 0.41%. This value is close to the International Target Values expressed by the IAEA for destructive analysis, making this a promising system for uranium IR measurements in general. The performance to date, on par with commercial quadrupole ICP-MS, was not anticipated. This work hopes to improve upon those characteristics by replacing the baseline Exactive instrument with a Q-Exactive Orbitrap mass analyzer. The Q-Exactive employs a quadrupole mass analyzer that can be used to pre-select the ions that are injected into the Orbitrap for high resolution analysis. It is expected that this added functionality will limit the number of interfering ions as well as any present matrix ions from entering the trap, thus extending the dynamic range and improving the accuracy and precision of the measurements.

Keywords: Atomic Spectroscopy, Isotope Ratio MS, Mass Spectrometry, Nuclear Analytical Applications
Application Code: Nuclear
Methodology Code: Mass Spectrometry
Carbon quantum dots (CQDs) have attracted enormous interest in recent years owing to their low cytotoxicity, excellent biocompatibility and strong photoluminescence. Prior research suggested that the structure of CQDs have significant influence on its photoluminescence and catalytic properties. Although some techniques, such as electron microscopes, X-ray photoelectron spectroscopy, Raman spectrometry, and Fourier-transform infrared spectroscopy have been employed to characterize the structure of CQDs, the detail information on the core and shell structures of CQDs are limited. This study aims to employ laser desorption/ionization mass spectrometry (LDI-MS) for analysis of CQD structures. We use three different CQDs, named CQD[CA], CQD[AC] and CQD[Spd], prepared from dry heating of citrate acid, ammonium citrate, and spermidine, respectively, as model CQDs to demonstrate our approach. The mass spectra of both CQD[CA] and CQD[AC] show core related anionic carbon cluster ions ([C\_n]^-, n = 3-10) with shot dependent increase in signal intensities. However, [CNO]^-, [CH\_3N\_2O\_3]^-, and [CH\_3N\_O\_4]^- MS signals in CQD[AC] samples are much stronger than [C\_n]^-, under fewer pulse laser shots. We suggest that the shot dependent increase in signal of [C\_n]^-. in both CQD[CA] and CQD[AC] is due to the pulse laser removing the surface functional group and then gradually fragment their carbon rich core. By contrast, we observed no [C\_n]^-. in CQD[Spd] samples.

This result is probably due to the large amount of nitrogen doped in its structures. Our study reveal LDI-MS is a simple method for the simultaneous analysis of core and surface structures of CQDs through control of pulsed laser irradiation.

Keywords: Analysis, Mass Spectrometry, Nanotechnology
There is great interest amongst archaeologists and conservators to understand the composition of ancient Peruvian dyes. Textiles, such as those from the Paracas burials, are often well preserved and show the use of more than 200 different colors. The Saltzman Collection is a notebook of South American dye recipes and samples prepared by UCLA physicist Max Saltzman in the 1970s. The collection comprises a number of different colors, including reds from three \[i\]Relbunium[/i] plant species and cochineal insects; a pink derived from \[i\]Opuntia[/i] seeds; four yellows derived from different plant species; and two plant tannin browns. The dye samples were prepared on wool from both sheep and alpaca, as well as cotton fibers both with and without mordants. This work describes our time-of-flight mass spectrometric analyses of the dyes on the protein fibers by a variety of different ionization techniques: direct analysis in real time (DART), electrospray and paper spray ionization (ESI and PS) and matrix-assisted inlet ionization (MAI). Each method provides different information about the dye compositions, with DART being the simplest as it requires no sample preparation. One drawback of the DART method is that it cannot provide information about the glycoside compounds present; however, both PS and MAI have the potential to provide more complete dye compositions with simple sample extraction.

**Keywords:** Art/Archaeology, Mass Spectrometry, Materials Characterization, Time of Flight MS

**Application Code:** Art/Archaeology

**Methodology Code:** Mass Spectrometry
Ceramic fragments found during excavations at Ferry Farm, which was home to the Washington family from 1738-1772, show evidence of having been mended with glue or cement before they were discarded. The glues available in the 18th century were prepared from a variety of recipes that included dairy products, resins, and collagen-based animal glues. A comparative database was generated for glues replicated from 18th-century recipes. Fragments of the historic residues were investigated by paper spray ionization and direct analysis in real time ionization (DART) mass spectrometry as well as Fourier transform infrared spectroscopy to determine their composition. Only traces of organic material remain in the historic glue samples. Amino acid analysis by DART-MS indicates that collagen may have been a part of the historic glue recipes. Additional mass spectrometry studies are ongoing to determine if peptides characteristic of casein or collagen are present to better identify the contents of the glues from Ferry Farm.
During the excavation of a Civil War period archaeological feature near Fredericksburg, Virginia in October 2015, a bundle of blue wool and other objects was recovered containing pins, buttons and a small glass bottle. The bottle showed evidence of the material it had once contained, possibly a medicinal preparation, which is today visible as a dark brownish solid. To determine the composition of the bottle contents, we undertook analysis of the material with Fourier transform infrared spectroscopy, direct analysis in real time mass spectrometry (DART-MS), and scanning electron microscopy with energy dispersive x-ray spectroscopy (SEM-EDS). Comparing the results of the analyses with period recipes for Civil War-era medicines, the composition of the residue was found to be consistent with the bottle having at one time contained a mixture of turpentine, mercury, and possibly animal fat. These components were commonly used in mercury ointments used in the treatment of skin lesions, particularly those caused by syphilis.

Keywords: Art/Archeology, FTIR, Mass Spectrometry, Mercury
Application Code: Art/Archeology
Methodology Code: Mass Spectrometry
The people of the Mill Creek culture lived near the Big Sioux and Little Sioux Rivers in northwest Iowa approximately 1000 years ago. Archaeological investigations have revealed many aspects of life in the Mill Creek villages, but questions remain regarding the extent of trade between Mill Creek and Mississippian people. Information about the trace element composition of the pottery can shed light on the origin of pottery fragments and help to answer these questions. In this study, 100 pottery sherds from six Mill Creek villages were analyzed by portable X-ray fluorescence (XRF) spectrometry. A total of 13 elements were determined with good precision, and four elements (zinc, zirconium, titanium, and chromium) were found to be particularly useful in distinguishing between the Big Sioux and Little Sioux villages. The results of this study are interpreted in terms of the proximity of the villages to one another and trading of pottery with the Mississippian culture.

Keywords: Art/Archaeology, X-ray Fluorescence
Application Code: Art/Archaeology
Methodology Code: X-ray Techniques
The Library of Congress has well over 10,000 wax cylinders, which are the first commercialized media for recording sound, in our collections. Unfortunately, these cylinders can have several conditions issues including cracking and exudation. To understand the cause of these issues and develop strategies to prevent further degradation, we are taking a multi-pronged approach to identify compositional changes resulting from aging, guide preservation of these materials, and to develop methods for cleaning cylinders for preservation and reformatting. To this end, we are performing a detailed chemical analysis of cylinders and fragments from our collection as well as recreating wax compositions guided by both primary literature and the results of our chemical analyses. By recreating compositions, a more detailed physical characterization of the materials may be made and any change in composition or degradation may be examined in detail.

Preliminary results have indicated that the inorganic composition of our wax cylinders is more complex than initial expectations that were based on formulations obtained from primary literature sources. The majority of the organic composition appears relatively consistent with reported formulations. Primary sources from the development of wax cylinders indicate that the identity of the metal soap in the compositions affect the material’s properties. We are currently replicating the observed wax compositions to shed light on the role metals play in the characteristics of the cylinder materials.

Keywords: Art/Archaeology, Material Science, Metals
Application Code: General Interest
Methodology Code: Chemical Methods
On August 5, 2016 contracted workers for the EPA near Silverton, CO. at the Gold King Mine, while performing maintenance to the tailing pond dam with heavy equipment, knocked the dam wall loose releasing three million gallons of contaminated waste water into Cement Creek, a tributary of the Animas River which is a tributary of the San Juan River. Following the release, a large yellow/orange plume was observed moving downriver through several agricultural communities of the San Juan, on the Navajo Reservation. The goal of this work is to quantify lead, arsenic, manganese, and uranium being deposited in the river sediment over time in the affected areas. Sample collection took place 3, 6, and 9 months after the spill. Sediment samples were collected in cleaned two foot PVC pipes. The top one inch of the core was taken for analysis as well as the bottom 1 inch for comparison. All samples were processed, acid digested completely by hydrofluoric digestion, and analyzed for trace metal composition by ICP-MS. Preliminary work has focused on the analysis of 15 of 95 samples for lead, manganese, uranium, and arsenic by ICP-MS. The preliminary results suggest the elemental levels to be near natural background concentrations, with lead concentrations increasing at sample locations over time. With the analysis of the next 80 samples, we expect to see total concentrations of lead, manganese, uranium, and arsenic in the samples and how these elements are depositing in the sediments and fields of these communities both spatially and temporally.

Keywords: Elemental Mass Spec, Environmental/Soils, ICP-MS, Lead
Application Code: Environmental
Methodology Code: Mass Spectrometry
A Bayou is a slow running (less than 0.5 cm per second) body of water often criss-crossing areas where industry and nature and human habitation occur such as found in Southwest Louisiana.

This work will focus on a number of studies throughout the last ten years, with emphasis on selected metals (determined by sample preparation by microwave followed by inductively coupled plasma-optical emission spectrometry (ICP-OES) in both pristine and polluted bayous. The work compares results of concentrations of metals from ten years ago through 2016/2017. Conclusions on results will be presented as well as results of ongoing studies.

Keywords: Atomic Emission Spectroscopy, Environmental, Environmental/Soils, Environmental/Water
Application Code: Environmental
Methodology Code: Atomic Spectroscopy/Elemental Analysis
Since early 1940’s, Southwest Louisiana has had a large petroleum refining and associated petrochemical industry. The area is also known for its recreational and commercial fishing. The overlap of these two areas caused pollution (natural and accidental) and was not seriously addressed until the early 1970’s with the introduction of legislation and a greater awareness of pollution control. This has lead to significant reduction in pollution but can continue to happen due to weather related causes (hurricanes) and accidents.

This work will focus on a number of studies (both in the field and in the laboratory) over the last fifteen year including selected metals in seafood (oysters and crawfish), waters and sediments, primarily using microwave sample preparation followed by inductively coupled plasma-optical emission spectrometry (ICP-OES) for metals and gas chromatography-mass spectrometry (GC-MS) for organics, in particular hexachloro di-enes.

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

**Application Code:** Environmental

**Methodology Code:** Atomic Spectroscopy/Elemental Analysis
Environmental Analysis of Metals

Comprehensive Water Survey of the State of Pennsylvania

Recent reports of water quality problems in Flint, Michigan have demonstrated the need for further water quality assessments across the United States. Locally, Pennsylvania water quality assessments are in dire need due to the state’s long history of industrial operations. Many factors can contribute to water quality problems such as conventional oil and gas development, acid mine drainage, and recently fracking. As a part of a comprehensive water quality assessment of organic and inorganic water parameters in nearly 100 samples taken across the state of Pennsylvania, we have analyzed for metal and trace element concentrations by inductively coupled plasma with atomic emission detection (ICP-AES). Among metals tested were Al, As, Ba, Cd, Cr, Fe, Pb, Ni, Sr, V, and Zn which can give diagnostic insight into a multitude of water quality and public health hazards. This comprehensive study can allow for a more complete picture of water problems across the state of Pennsylvania.

Keywords: Geochemistry, ICP, Metals, Water
Application Code: Environmental
Methodology Code: Atomic Spectroscopy/Elemental Analysis
Iron–barium coprecipitation phenomenon coupled with gross beta measurement for strontium 90 was demonstrated using sea water sample as a rapid radiometric analysis. The sea water samples were collected within 2 km around Fukushima Dai-ichi nuclear power station at the term from October 2011 to March 2012. In the measurement of strontium 90, a low-background gas flow counter (Geiger-Müller counter tube; LBC) was used. In this presentation, we would like present that a gross beta measurement with iron–barium coprecipitation method was very effective as compared to traditional milking process with LBC. In the investigation, the quantitative values between using gross beta measurement method and the official method (traditional milking-LBC method) were shown a good correlation. Thus it was found that the means using gross beta radioactivity measurement can be quantify the strontium 90. The whole process need a time for 12 hours to quantify strontium 90 and the time is 1/40 shorten as compared with traditional milking-LBC method. Furthermore, by measuring the time-course of signal decay using yttrium-90 (half-life 64 hours), it was possible to prevent misrecognition with other nuclides which emit beta-ray. Accordingly, an iron–barium coprecipitation phenomenon coupled with gross beta measurement method for quantification of strontium 90 is effective to measure sea water as a screening method and it will be helpful as one of the monitoring means of strontium-90 measurement.

Keywords: Environmental Analysis, Environmental/Water, Radiochemical Methods
Application Code: Environmental
Methodology Code: Process Analytical Techniques
Previously, our group developed an electrochemical instrument equipped with a gold electrode capable of performing anodic stripping coulometry (ASC) for the detection of arsenite at trace levels in water. A critical aspect in this analysis is establishing a precise volume so that Faraday’s law, \( Q = nF\Delta, \) can be utilized for quantification of analyte. In this work, several methods are tested to perfect the construction of a device (referred to as the cell) with a defined volume. One technique, amongst others, utilizes microfabrication to build the device analysis chamber directly on top of the working electrode using SU-8 photoresist. Cells of particular SU-8 height, and thus exact volume, were fabricated by varying parameters during photolithography. Then, to enhance the device further, the cell is being interfaced with a platform which will allow for deployment of the device as a remote, autonomous instrument for heavy metal analysis. Remote sensing is advantageous because it enables continuous electrochemical measurements to be performed on-site, in real time. The platform provides automated sample intake, electrochemical analysis, and wireless data communication in a matter of a few minutes and will eventually incorporate sample pretreatment, such as filtration, deoxygenation, and acidification. By combining the fixed volume sensor with the platform for remote operation, a more practical instrument has been created that is affordable (~$600), mass-producible, and well suited for investigating additional applications that involve other trace metals, including cadmium, lead, and mercury, as well as new electrode materials, such as boron-doped diamond.

Keywords: Electrochemistry, Environmental Analysis, Metals, Sensors
Application Code: Environmental
Methodology Code: Sensors
At present large areas devoted to agricultural purposes are polluted by either heavy metals and organic micropollutants such as PCDD/Fs and PCBs. Rapid detection of heavy metals in soil can be obtained by field portable metal analyzer such as X-Ray Fluorescence (FP-XRF). EPA Method 6200 describes the FP-XRF procedure: it allows identifying hot spots where to focus the collection of soil samples to be analyzed by traditional techniques. Instead, the analysis of the organic micropollutants are still very expensive and time consuming posing a serious challenge in the determination of the pollution in large areas such as agricultural soils due to the large number of sample to be analyzed.

The aim of the present study has been to find an analytical procedure to estimate unknown concentrations of PCDD/Fs and PCBs on the grounds of statistical relationships between the organic micropollutants and metal values measured by FP-XRF by means of an Artificial Neural Network (ANN) data modellization.

The initial phase of the method involves the analysis of a consistent number of samples by FP-XRF and HRGC/HRMS (organics): their concentrations are used to train an ANN model. In a secondary phase, a large number of samples are analysed by FP-XRF only while the above-mentioned ANN model estimates the organic compounds. If their concentrations identify a hot spot, confirmatory analysis will be performed by HRGC/MS. The results of the confirmatory tests will be used to train again the neural network, improving model accuracy at each repetition of the procedure.

As an example, the procedure has been applied to an Italian agricultural soil contaminated by Cu, As, Zn, Pb, Mn and PCBs. The trained network was able to correctly predict 86.2% of the PCBs values, with a validation set RE of 0.922 and a RMSE of 0.026. Despite the absence of a linear relationship between input and output variables, the ANN model has shown a good level of reliability.

**Keywords:** Chemometrics, Environmental/Soils, Neural Network, Statistical Data Analysis

**Application Code:** Environmental

**Methodology Code:** Chemometrics
Colorimetric spot test analysis is a powerful tool for rapid qualitative/quantitative analysis and can be applied in situations in which a decision is critical, but sophisticated analytical instrumentation or time consuming procedures cannot be used [1,2]. In this study, we report the development of a portable, low-cost, high-throughput visual colorimetric paper-based forensic oriented sensor for the detection of phenacetin (PHE) adulteration in seized cocaine samples. The method consists in the use of white office paper as substrate and wax printing technology [3] to fabricate detection zones. Under the optimized experimental conditions, a linear analytical curve was obtained for phenacetin concentrations ranging from 0 to 730 µmol L⁻¹, Fig. 1. Linearity was observed from 0 to 360 µmol L⁻¹, and the straight line was in accordance with the equation: (Magenta percentage color) = 1.19 + 82.1 (CPHE / mmol L⁻¹), R² = 0.990. The limit of detection was calculated as 20 µmol L⁻¹ (3/slope). The accuracy of the proposed method was evaluated using real seized cocaine samples, and an addition and recovery protocol. Financial support: FAPESP, CNPQ and CAPES.

Fig.1-A) Photograph of the colorimetric response of phenacetin at different concentrations ranging from 0 to 0.73 mmol L⁻¹ in triplicate under the optimum analytical parameters. B) Analytical curve obtained using the magenta percentage for different phenacetin concentrations.

References:

Keywords: Forensic Chemistry, Lab-on-a-Chip/Microfluidics, Portable Instruments, Sensors
Application Code: Homeland Security/Forensics
Methodology Code: Sensors
Fentanyl analogs and designer opioid drugs are a hot topic in the news right now contributing to numerous fatal overdoses. These drugs elicit analgesic effects similar to heroin making them desirable drugs to abuse. Fentanyl analogs and designer opioid drugs are expected to be more prominent in forensic casework in the near future. Fentanyl analogs and designer opioid drugs can be seen in forensic casework either alone or can be mixed with other drugs of abuse such as heroin. It is therefore necessary to have an efficient methodology to identify these compounds. Currently, Gas Chromatography-Mass Spectrometry (GC-MS) is used to identify drugs of abuse and is considered the “gold standard” in forensic casework. However, analysis times can often range from 15-60 minutes in length. Another drawback to GC-MS is need for spectra library matching, giving the need for analytical reference materials for identification leading to an inability to identify new designer drugs before a reference material is available. In this study, Direct Sample Analysis Time-of-Flight Mass Spectrometry (DSA-TOFMS) was utilized to provide rapid identification of fentanyl and related synthetic opiates. DSA is a direct ambient ionization source, requiring no chromatography and minimal sample preparation. High resolution time of flight mass spectrometry generates empirical formula information bypassing the need for a reference material, and in-source collisionally induced dissociation (CID) produces additional structural information for confirmation. An overview of the instrumentation and use of DSA-TOFMS to rapidly generate exact mass data and fragmentation data from in-source CID for the identification of synthetic opiates will be presented. Analytes explored include: heroin, 6-monoacetylmorphine, morphine, fentanyl, acetyl fentanyl, butyryl fentanyl, furanyl fentanyl, U-47700, and W-18.

Keywords: Forensics, Forensic Chemistry, Mass Spectrometry, Time of Flight MS
Application Code: Homeland Security/Forensics
Methodology Code: Mass Spectrometry
N-(methoxybenzyl)-phenethylamine compounds (also known as NBOMes) are an emerging threat to public health with fatality related investigations increasing throughout the country. NBOMes obtained as legal highs through unregulated internet purchase are typically consumed on blotter paper. The user experience has been described as a more potent, but similar high when compared to lysergic acid diethylamide (LSD). Previous reports from this group have demonstrated the utility of non-targeted high resolution mass spectrometry platforms for this application paired with both ambient ionization and electrospray ionization sources since authentic case work samples are often obtained as mixtures of true unknowns (reported at MAFS and NEAFS 2015). This work employed a minimal sample preparation via a 5 min liquid extraction of the active from the paper. In an extension of this work, chemical imaging by FTIR microscopy was evaluated to provide an orthogonal workflow solution for confirmation to leverage the advantage of DSA-TOFMS for screening (e.g. speed of analysis, reduced cost in consumables and waste etc.). Method development variables considered included variation in sampling depth and scan modes to generate a spatial chemical map of the sample. This work was completed in collaboration with the State of Maine Department of Health and Human Services (Augusta, Maine), Illinois State University (Normal, IL) and Boston University School of Medicine Department of Anatomy & Neurobiology (Boston, Massachusetts). It is anticipated that this no-prep workflow would be of broad-based interest to the forensic community on the topic of emerging drugs of abuse, designer and synthetic drugs.

Abstract Text

Keywords: Forensics, FTIR, Imaging, Microspectroscopy
Application Code: Homeland Security/Forensics
Methodology Code: Surface Analysis/Imaging
Identification of Volatile Organic Compounds Present within a C4 Storage Magazine and Emitted by C4: Using High-Volume Sampling (HVS) Traps that are Extracted into Thermal Desorption (TD) Tubes for TD-GC/MS Analysis

Inho Cho  
TSA

The interior of an explosive magazine is lined with three inches of kiln dry wood (mainly pine) and plywood; the exterior of the magazine is ¼ inch thick sheet metal. In this study, volatile organic compound(s) (VOCs), which are contained within the magazine’s interior, and from the explosives therein enclosed were identified. High-volume sampler (HVS) traps and commercial thermal desorption (TD) tubes were used to collect VOCs from the magazine’s inside and a headspace glass vessel (HSGV) was used to collect headspace samples of the explosive into a TD tube. The HVS traps’ and tubes’ samples were analyzed on the Thermal Desorption System-Gas Chromatography/Mass Spectrometry (TDS-GC/MS) - a Gerstel TDS cryogenic system with an Agilent 5975 C MS.

The HVS front-end collection device is a novel sampling technique and it has been developed for Homeland Security applications to harvest VOCs from hidden illicit materials in 30 seconds. Most of the commercial TD tubes require long sample collection times of 2 + hours. A high static pressure commercial vacuum system was applied to collect the VOCs from the magazine onto an HVS trap. The sampled HVS trap was thermally extracted into a TD tube for the TD-GC/MS analysis using a custom-made HVS trap desorber.

Approximately, 70 VOCs were identified from the inside of the magazine at ambient temperature and humidity during summer months in New Jersey. Two C4 headspace samples were harvested into TD tubes; one was bare C4, from which the original factory wrapper was removed and the other one was C4 in its original condition, retaining its wrapper. Mainly, alkyl aldehydes were found from the magazine and cyclohexanone, 2-ethyl-1-hexanol, butyl ester acetic acid, acetic acid, and 2,3-dimethyl-2,3- dinitrobutane were identified from the tagged C4; however, butylated hydroxytoluene was found exclusively from the C4 with its factory wrapper.

Keywords: Adsorption, Forensics, Gas Chromatography/Mass Spectrometry, Thermal Desorption
Application Code: Homeland Security/Forensics
Methodology Code: Gas Chromatography/Mass Spectrometry
In this project, colorimetric tests have been implemented on paper microfluidic devices permitting detection of metallic and organic residues from low explosives. These devices are prepared from chromatographic paper using hydrophilic channels created using wax printing. Capillary action then mobilizes liquids containing dissolved analytes through these channels permitting multiplexed detection. To determine each individual analyte, colorimetric reagents are placed at the terminal end of each channel. Because the reagents are dried on the device prior to use, shelf lives are prolonged when compared to liquid reagents. We have been exploring forensic applications of this technology.

Low explosives commonly consist of mixtures of oxidizers and fuels. They are of great concern as they can be used in improvised explosive devices. For example, aluminum fuels and nitrate-based oxidizers are commonly utilized in flash powders and can be detected using an aluminon test that produces a red metal complex, while the nitrate oxidizer can be determined using the Greiss reagent. Additional multiplexed tests have been developed by adapting other colorimetric reagents to the paper format. The key issue to be addressed with these devices is compatibility of the analytes with the flow-based system. Detected samples can be read by eye or ImageJ software. Once produced, the paper-based devices are compact and inexpensive, producing a result in less than five minutes. They should be of great use to investigators for the presumptive detection of a variety of explosives residues in the field.

Keywords: Detection, Forensic Chemistry, Lab-on-a-Chip/Microfluidics, Trace Analysis
Application Code: Homeland Security/Forensics
Methodology Code: Microfluidics/Lab-on-a-Chip
Forensics and Homeland Security

Handheld High Pressure Mass Spectrometry with a Novel APCI Dual-Polarity Source for Threat Detection

There are many missions across the military, law enforcement, and first responder domains that can benefit from reliable, on the ground chemical measurements. Chemical measurements performed in the field however are constrained by many factors such as the need for high sample throughput, high confidence in the analysis result, and low limits of detection. Historically, mass spectrometers with ambient ionization sources readily meet these requirements but the need to operate at high vacuum imposes severe penalties on power/battery, start-up time, cost and simplicity of operation & maintenance. The recent emergence of mass spectrometers capable of operating at much higher pressures has offered an alternative free of these high-vacuum constraints. Instruments are now capable of performing mass analysis at operating pressures around 1 torr, a pressure readily maintained by miniature scroll pumps, obviating the need for high vacuum (turbo) pumping capability. In this talk we’ll review the foundations of the high-pressure mass spectrometer, a dual-polarity APCI source approach and the resulting analytical advantages afforded by this configuration.

Keywords: Analysis, Chemical, Ion Trap, Mass Spectrometry
Application Code: Homeland Security/Forensics
Methodology Code: Mass Spectrometry
In forensic evidence analysis, mass spectrometry (MS) is widely known for its strong discriminating power. Translating large, lab-bound instruments to smaller, portable units with similar performance is currently being sought out as a novel method in accelerating the throughput of evidence processing. Ambient sampling, portable MS systems have shown promise in this pursuit, simplifying both sample preparation and manipulation and assisting field operators in obtaining quick, accurate identification. In regards to new technologies, the burden of determining admissibility in legal proceedings is placed on the presiding judge, with the Daubert Standard being what guides most jurisdictions in determining the credibility of a method. The Daubert standard, stemming from Daubert v. Merrell Dow Pharmaceuticals, 509 U.S. 579 (1993), outlines main guidelines for decision-making: (1) can the technique can be tested and/or refuted? (2) has it been subjected to peer review? (3) what is potential/known rate of error? (4) are there standards and/or controls for using the technique? (5) what is the degree of acceptance in the relevant scientific community?

In this work, we have attempted to assess whether the FLIR Systems AI-MS 1.2, a ruggedized, portable cylindrical ion trap MS with the ability to be coupled with ambient ionization methods like paper spray ionization source (PSI), has the potential to hold up against the Daubert Standard. Literature review from legal, scientific and governmental databases was conducted, generating citation numbers of publications, grant proposals, as well as court cases in which portable MS was being explored. Error rate was determined to be characteristically low, collected using large datasets. Further, the proposed methods of operation were scrutinized according to the Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) recommendations to show adherence to accepted standards.

Keywords: Forensic Chemistry, Mass Spectrometry, Portable Instruments
Application Code: Homeland Security/Forensics
Methodology Code: Mass Spectrometry
Forensics and Homeland Security

On-Site Determination of Chemical Warfare Agents by Handheld Raman Analyzer with 1064 nm Excitation Laser

In the cases of chemical warfare/terrorism, it is desired for first responders to be able to identify chemical warfare agents (CWAs) in suspicious on-site samples rapidly and easily, in a non-destructive manner. When samples are contained in glass or plastic bag, it is strongly preferable for responders to be able to analyze the samples without opening the container. Various field technologies are adopted for screening hazardous materials in cases of suspected terrorism cases. Portable FT-IR and Raman spectroscopic instruments are representative examples. However, these technologies suffer from serious interference by the sample matrix such as water for FT-IR and fluorescent compounds in the case of Raman. Previously, we examined the portable Raman analyzer Xantus-2 (Rigaku Corporation) with selectable two laser excitation wavelengths of 785 nm and 1064 nm (or 532 nm). Here, we investigated the usefulness of the new handheld Raman analyzer Progeny ResQ (Rigaku Corporation) with a laser excitation wavelength of 1064 nm. Laser power and exposure time are automatically set depending on the sample conditions. The authentic CWAs examined were liquid nerve gases (sarin, soman, cyclohexylsarin, ethylsarin, isobutylsarin, VX, Russian VX), liquid blister agents (mustard gas, lewisite 1, nitrogen mustard 1, 2, 3), solid tear gases (2-chloroacetophenone, o-chlorobenzylidene malononitrile) and solid vomit agents (diphenylchloroarsine, adamsite (crude preparation)). Although clear Raman spectra could not observed for nitrogen mustard 3 nor adamsite by portable Raman spectrometer with 785 nm excitation laser, characteristic spectra could be obtained for all the CWAs examined by ResQ. A search using the default library on ResQ indicated that nine CWAs were correctly identified. The remaining CWAs were not registered in default library.

Keywords: Detection, Forensic Chemistry, Raman Spectroscopy
Application Code: Homeland Security/Forensics
Methodology Code: Vibrational Spectroscopy
In cases of chemical warfare/terrorism, it is desired to detect chemical warfare agents (CWAs) in the field. We have investigated the detection performance of various kinds of portable ion mobility spectrometry (IMS) equipment for detecting CWAs. In this presentation, we examined two types of IMS instruments, one with $^{63}$Ni ionization and no dopant (Smiths Detection, MMTD-DT-1) and another with corona discharge ionization and ammonia dopant (Smiths Detection, LCD 3.3), and compared their ion mobility behaviors, considering reduced ion mobility constants (Ko) with molecular assignment. In MMTD-DT-1, G-type nerve gases provided positive dimer-related ions. V-type nerve gases and nitrogen mustards provided positive monomer-related ions. Mustard gas (HD), Lewisite 1 (L1), nitrogen mustards, phosgene (CG) and chloropicrin (PS) provided negative chloride-related ion and negative Ko 1.59 ion. Hydrogen cyanide (AC) and chlorine (CL) provided negative cyanide-related and chlorine-related ions, respectively. The concentration-dependent sarin ion peak transition was observed. As sarin level was increased, dimer-related ion was shifted to hydrated dimer-related ion and trimer-related ion. In LCD 3.3, G-type nerve gases provided positive monomer-related and dimer-related ions. V-type nerve gases and nitrogen mustards provided positive monomer-related ions. Their ions were assumed to be conjugated with ammonia. HD, L1, AC and CL provided negative monomer-related ions. Cyanogen chloride, CG and PS provided chloride-related ion. The concentration-dependent sarin ion peak transition was also observed. As sarin level was increased, monomer-related ion was shifted to dimer-related ion. In conclusion, ion mobility behaviors were differed between two IMS instruments with different ionization and dopant system.
In the forensic discrimination of glass fragments by elemental analysis using ICP-MS, the more numbers of matching elements can provide the more evidential value in terms of a common origin of glass fragments comparison. The purpose of this study is to allow accurate determination of Ti and Fe in glass fragments using reaction cell ICP-MS/MS by reducing the interferences from $^{48}\text{Ca}^{+}$ and $^{40}\text{Ar}^{16}\text{O}^{+}$ on $^{48}\text{Ti}^{+}$ and $^{56}\text{Fe}^{+}$, respectively. Sheets of glass produced at the same manufacturing line and a glass standard reference material were used as samples. Glass fragments (2 mg) were dissolved in 600 [micro]L of mixture containing HF : HCl : HNO$_3$ (2 : 1 : 1) by 2 h of ultrasonication, followed by heating at 80 [degree]C until the samples were completely dried. The samples were reconstituted using 800 [micro]L of 4 M HNO$_3$ and 700 [micro]L of deionized water, and then left over a night. After 2.5 mL of deionized water was added and mixed, Ti and Fe in the solution were analyzed by ICP-MS/MS (ms/ms mode) using O$_2$ for Ti and H$_2$ for Fe as reaction gas. Measured (certified) values for Ti and Fe in the standard were 47.8 (45.2-50.4) [micro]g g$^{-1}$ (50.8 (50.0-51.6) [micro]g g$^{-1}$) and 50.8 (47.5-54.1) [micro]g g$^{-1}$ (51 (49-53) [micro]g g$^{-1}$). Some pairs of glass produced at the same line exhibited significantly different concentrations of Ti and also Fe, indicating that this method is effective for the discrimination of glass fragments.
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**Abstract Text**

Currently, the most common field detections for cocaine is immunoassay. However these tests can produce the false positive results due to the environmental contaminations. We have been working on an alternative platform for AuNPs/aptamer detection based on paper microfluidic devices to improve the detections for cocaine. A chip design has been created with AuNPs and aptamers to a ready-to-use format. The devices can be used at crime scenes, in laboratories and any other locations where the suspected powders may occur. The paper chips are easy to prepare and inexpensive to operate. Furthermore, they can be conveniently stored for later uses.

The paper microfluidic devices are prepared using a wax-ink printer, thermal laminator, chromatography paper, aptamers and gold nanoparticles. The wax-ink printer and a thermal laminator produce hydrophilic channels defined by melted wax on the paper. Next gold nanoparticles and aptamers are prepared for the channel. Cocaine samples in acid/basic form are dissolved in solutions and then transferred to the chips. Cocaine next travels down the channel via capillary action, interacting with the aptimers and causing a color change to occur due to the aggregation of the nanoparticles. When cocaine is not present the nanoparticles cannot aggregate and no color change occurs as aptimers are then free to bind gold. The entire process takes ~5 minutes. The applied aptamer is specific for cocaine. This poster reports a preliminary validation of this device including tests for sensitivity, specificity and stability against a variety of potential interferences.

**Keywords:** Biosensors, Detection, Nanotechnology

**Application Code:** Other

**Methodology Code:** Sensors
Triton Systems Inc., Chelmsford, MA has developed a universal CWA, TICs, explosives and narcotics vapor preconcentrator to increase the sensitivity of chemical detectors at the point of analysis and to reduce the influence of ambient interferents.

As an adsorption media the device utilizes fibers coated with a thermally stable polymer having a tuned hydrophobicity/hydrophilicity ratio. The sampling system employs two miniature pumps for air sampling and vapor release. It also contains a novel, low power consumption heater capable of heating the 0.1 g preconcentrator substrate to 250°C within two (2) seconds using 20 Joules. The product is being marketed under the Traceptor trade name. It has a microcontroller, a user-friendly display for setting sampling and release parameters, and also for hardware status indication. The palm-sized portable device weighs a total of 550 g, and can provide up to 200 sampling/heating/release cycles on one battery charge.

The technology was designed for use with a wide range of chemical detectors, though initial adoption is anticipated with analyte-ionization based detectors. Preconcentrated chemicals are released as a dense cloud of vapors in a 2 seconds pulse concentrated in a few cubic centimeters volume. The same preconcentrator substrate can be used many times for chemical sorption/desorption cycles, and when loaded with analytes can be stored for prolonged periods of time.

The preconcentrator technology has been tested with a number of chemical detectors, including MS, IMS and PID and has demonstrated the ability to increase the detector’s sensitivity by a factor of 30 to 1500, depending on the nature and concentration of the analyte and sampling conditions.
Laser speckle is the random interference pattern observed due to diffuse reflection of different parts of a coherent beam from different spots on the surface, which interfere with random amplitudes and phases on a camera. Since it is an interference phenomena, the speckle pattern observed is highly sensitive to the exact morphology of the surface and small perturbations. One new type of absorption spectroscopy recently developed by Stolyarov et al. (Optics Express, vol. 40(24), pp. 5786-5789, 2015.) is photothermal speckle modulation. In the technique, a visible laser beam illuminates a surface with a material-of-interest on it. The reflected light is then imaged with a camera. A second modulated laser that is absorbed by the material of interest will modulate the speckle pattern due to thermal expansion of materials on the substrate. This technique can be used to perform absorption spectroscopy since the intensity of the speckle modulation will depend on the magnitude of the absorption of the material that is illuminated. One advantage of this technique is that a modulated infrared beam can be used to probe the vibrational modes of the sample, which are very distinctive for determining chemical identity, while the speckle beam can be a visible beam that is easily detected using inexpensive cameras. The application of photothermal speckle modulation to chemical detection will be discussed. Some topics that will be addressed include photodegradation, saturation, and the potential for use in hyperspectral imaging.
The development of renewable energy is a major challenge all over the world, due to various environmental, geopolitical and economic issues. Biomethane to be injected into the grid is one answer. The French Energy Agency built a roadmap for biomethane in France. The scenario predicts by 2030 a production of 30 TWh which equals to about 1400 biomethane injection plants.

According to French specifications, a number of parameters of biomethane need to be monitored before the injection into the gas grid such as Calorific Value or trace compounds (e.g. water content, ammonia, sulfurs, mercury, chloride, etc.). Nevertheless, others trace components could be present in the gas. Thus additional measurements are necessary in order to ensure end users safety and grid integrity.

During biogas and biomethane production processes (biogas from agricultural waste, household waste or sludges from WWTP), different Volatil Organic Compounds can be found in the such as siloxanes, terpenes, alcohols, ketones, etc.

ENGIE LAB CRIGEN has developed an efficient analytical strategy based on the use of both thermal desorption unit (TDS) and a gas chromatograph coupled with a mass spectrometer (GC-MS). Thanks to the use of this specific detector, the analytical method enables the identification and quantification of more than a hundred components in one sample. The sampling system is used to preconcentrate trace compounds and leads to quantification limits of 1 µg/scm. It is an easy-to-use technique which matches well with constraints on site. ENGIE has assessed the online sampling method.

The use of thermal desorption and GC-MS allows to combine an easy-to-use sampling technique and a powerful laboratory device. A global characterization of trace components of a sample can be accessed quickly in a one-shot operation. Building on this success, this strategy is now used by ENGIE LAB CRIGEN to characterize real biomethane samples on production sites.

**Keywords:** Biofuels, Energy, GC-MS, Thermal Desorption

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

**Methodology Code:** Gas Chromatography/Mass Spectrometry
In response to environmental, geopolitical and economical issues, one of the great worldwide challenges lies in the use of Renewable Energies. The Bioenergy industry is going to transform the Gas industry. From diverse biomasses-transformation processes, new categories of gases are appearing: Biogas, Syngas, Biomethane,...

This diversification of sources bring with it a new gas quality (variety of chemical compounds and concentrations) which render the situation much more complex. 2D GC appears to be a fast and straightforward analytical tool enabling Enhanced Diagnostics for Gas and Bioenergy Industries. The 2D GC allows the analysis of components that previously were difficult to separate in samples with complex matrices.

Biogas, Syngas, etc. and associated condensates are new complex matrices due to the large diversity of their possible sources and production pathways. During green gases production processes, a large scale of trace compounds could be present and need to be characterized. A one-shot and sensitive characterization is essential because it is efficient and less time-consuming when compared to a multi-technical approach.

With the collaboration of ESPCI, ENGIE LAB CRIGEN has developed methods that enable an overall characterization of trace compounds in green gases samples. The benefits of 2D GC are numerous. Technically 2D GC leads to an increase of peak capacity (x2-3 more than in 1D GC), a higher sensitivity and lower limit of quantification (x3 more than in 1D GC). This analytical tool allows a better knowledge of gas and effluent compositions. Different applications are available: snapshot fingerprint, monitoring of compounds of interest, gas origin recognition.

In the context of the new Gas and Bioenergy Industry, 2D GC may allow better and faster diagnostics for process performances and risks management. In this study ENGIE Lab CRIGEN – in collaboration with ESPCI – has highlighted that 2D GC may be a perfect tool to characterize new gases.
Government-subsidized farm fuels are often dyed to distinguish them from consumer diesels. “Fuel-washing,” where dye is removed from fuel that is subsequently resold at a higher price, has been a major concern for many, tax, regulatory, and law enforcement agencies. Accutrace S10, a new fuel marker developed for use in the UK, is more difficult to remove and can be detected at low concentrations to prevent dilution and illegal reselling of fuel. According to the UK agency HMRC (Her Majesty's Revenue and Customs), advanced fuel marker analyzers should be able to quantitate the fuel marker in diesels that have been diluted by up to a factor of 100. Without the need for any additional separation methods or sample preparation such as heart-cutting or further distillation, spiked diesel samples were analyzed in less-than-15-minute runs that allowed for quantitation in the concentration range of 2500ppb-10ppb, corresponding to ranges of 100% subsidized fuel to less than 1% subsidized fuel and exceeding the HRMC targeted quantitation range on the Pegasus-BT. This quick analysis that is both simple and quantitative is an excellent addition to the arsenal in the war against fuel-washing.
Fuel type is specified by engine manufacturers for proper engine performance and to prevent engine damage or failure. Cross contamination of fuels can occur from shared fuel delivery pumps, carry over from fuel storage tanks, fuel theft with substitution of cheaper alternate fuels, human error and even sabotage. Identification of wrong or contaminated fuel can influence engine warranty.

Rapid low resolution gas chromatography is used with existing hardware configuration of ASTM D7593 for fuel dilution in used engine oil. Minor method modifications allow for the rapid differentiation of fuel types in the fuel supply in 4 minute analysis.

Common interpretations of the fuel analysis include gasoline in diesel, diesel in gasoline, biodiesel in diesel, kerosene in gasoline, kerosene in diesel and methanol or ethanol in gasoline.

Keywords: Fuels\Energy\Petrochemical, Gas Chromatography, GC
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Gas Chromatography
Analysis of Sulfur Compounds in Light Petroleum Liquids by Gas Chromatography and Pulsed Flame Photometric Detection (PFPD) Using ASTM Method D5623

ASTM D5623 is a method primarily for the determination of volatile sulfur-containing compounds in light petroleum liquids with a final boiling point of 230°C or lower at atmospheric pressure, such as petroleum distillates and gasoline. Many sulfur compounds in light petroleum liquids are odorous, can cause corrosion to equipment, inhibit or destroy catalysts, and ultimately have a negative impact on product costs and quality. Sulfur in fuels also causes air pollution. The ability to speciate sulfur compounds is useful in controlling sulfur compounds in finished products and is often more important than determining total sulfur content alone. Gas chromatography combined with a Pulsed Flame Photometric Detector (PFPD) can provide a reliable means to identify and quantify sulfur compounds in petroleum liquids. Other sulfur selective detectors may be used for ASTM D5623 but the PFPD offers several advantages. The PFPD has improved sulfur selectivity from hydrocarbon matrices, increased sensitivity, and equimolar sulfur response. The equimolar response allows quantitation of unknown sulfur compounds that may be present in samples. This study will demonstrate the use of a new generation PFPD for the analysis of light petroleum liquids following the current ASTM D5623. A robust, yet sensitive method is needed for both total and speciated sulfur especially in light of the rapid approach of USEPA Tier 3 requirements in 2017. This rule sets new vehicle emissions standards and lowers the sulfur content in gasoline to 10ppm. The Tier 3 gasoline sulfur standards are similar to levels already being achieved in California, Europe, Japan, and several other countries. System performance criteria will be met and calibration will be performed according to ASTM D 5623. A representative list of sulfur compounds will be analyzed. A survey of local gasoline samples will also be performed.

Keywords: Fuels, Energy, Petrochemical, Gas Chromatography, Gasoline, Sulfur
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Gas Chromatography
Permanent gas analysis is routinely performed in a variety of industries to include petrochemical. In petroleum applications standard analyses consist of a complex system of packed columns and numerous valve switching timed events. In this presentation we will illustrate a simple solution for the analysis of permanent gases using a dual column system utilizing TCD, FID/Methanizer detection. The Methanizer consists of a Ni-catalyst in a heated chamber that converts CO and/or CO2 in the presence of hydrogen, into a FID detectable gas, methane. It is a modern, user friendly and accurately controlled device that can be easily integrated into any existing GC/FID system. Examples of analytical solutions, robustness of the unit, limit of detection and system performance will be highlighted.
Liquefied Petroleum Gas (LPG) is used as an alternative to natural gas, gasoline, aerosol propellants, and refrigerants. LPG is a mixture of hydrocarbons mainly comprised of butane or propane. Other hydrocarbons such as ethane, propylene, butylene, and pentane are found in LPG samples at lower quantities. During the production of LPG, fast and accurate analytical results are vital to the production stream. Using the proven Microelectromechanical (MEMS) technology of micro GC, analysis time is significantly faster when compared with the ASTM D2163-07 standardized method. Depending on the application, Micro GC Fusion is coupled with a flow through vaporizer or a fixed volume vaporizer for enhanced repeatability and uniform vaporization. The modular design, coupled with temperature programming allows the new Micro GC Fusion to provide sharper peaks, faster analysis, and greater separation when analyzing LPG samples.
Exploration and production of natural gas (NG) from unconventional reservoirs demands development of new monitoring techniques. Raman spectroscopy can deliver information in a non-invasive and quick way and it can be considered as a promising tool for determination of natural gas composition.

This work presents a specially designed laboratory Raman system for qualitative and quantitative analysis of natural gas (NG). Tested samples contain different ratios of following components: methane, ethane, propane, n-butane, iso-butane, n-pentane, iso-pentane, n-hexane and nitrogen. The apparatus is equipped with a specially designed high pressure and high temperature (HPHT) autoclave which allows to perform measurements in simulated in-situ conditions. For raw data analysis, machine learning techniques were applied to determine the percentage concentration of each component in the given mixture — it is a starting point for determination of molecular content of natural gas components in two-phase systems. Challenges in developing such analyzer range from autoclave design, laser beam path, Raman system calibration issues, gas mixture preparations, fluorescence, fiber optic losses and algorithm calibration. The paper gives an overview of the most recent achievements and the work in progress.

Acknowledgments: This work was performed with financial support from the National Centre for Research and Development and industrial partners, project no.: BG1/IRES/13.

Keywords: Gas, Hydrocarbons, Quantitative, Raman Spectroscopy

Application Code: Fuels, Energy and Petrochemical

Methodology Code: Vibrational Spectroscopy
Lubricating oils are complex mixtures composed of a base oil along with anti-oxidants and other additive packages. Important properties of these oils include the ability to dissipate heat, lubricate and withstand oxidation at service temperatures. Differential Scanning Calorimetry (DSC) and Pressure Differential Scanning Calorimetry (PDSC) are two instrumental techniques which have been found useful in evaluating Oil Service Life at elevated temperatures, characterizing oil formulations and screening of anti-oxidants.

The DSC allows for the measurement of the Oxidation Induction Time (OIT) as an accelerated test that can be performed to give a qualitative evaluation of the oxidative stability of a material. This time can be used as a measure of the thermal stability of lubricants in an oxidative atmosphere.

Further experiments and data analysis can provide an estimation of the remaining useful life (RUL) of a lubricant. Data will be demonstrated showing the utility and advantages of the DSC methods.

Keywords: DSC, Fuels\Energy\Petrochemical, Material Science, Thermal Analysis
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Thermal Analysis
Fourier Transform Infrared (FT-IR) spectroscopy is one of the primary analytical techniques for monitoring the degradation of in-service lubricant oils, offering fast analysis times and automation for high sample throughput. The infrared spectrum contains a wealth of information about the formation of degradation products as the oil ages and the presence of contaminants. Greases undergo similar degradation processes, such as oxidation and additive depletion, but chemical studies of in-service greases are significantly more challenging than for the lubricating oils due to the grease matrix.

Studies of greases by FT-IR have been limited to qualitative identification of components or semi-quantitative measurements due to the limited sampling choices for the grease matrix. Attenuated Total Reflectance (ATR) offers a fast and easy method for obtaining FT-IR spectra of greases. However, the effective pathlength is in the order of a few microns, leading to weak IR spectra and an inability to measure small changes in the chemistry of the grease. Transmission measurements by capillary film between salt windows also leads to short pathlengths of inconsistent and unknown values.

FT-IR studies of greases using a novel transmission sampling accessory are shown, benefitting from ease of sampling at fixed pathlengths. Cell material and pathlength options allow for the optimization of the FT-IR measurement and for quantitative measurements. This sampling method offers significant advantages over ATR measurements, with typical, highly reproducible pathlengths of 50 or 100 microns, allowing the detection of small changes within the grease.

Keywords: FTIR, Material Science, Petrochemical, Sample Preparation
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Vibrational Spectroscopy
In the last decade, Pair Distribution Function (PDF) analysis has transformed from a niche method to a broadly useful tool capable of addressing complex problems in battery research. PDF probes local structure with crystallographic-resolution, but is not limited to crystalline materials. Accordingly PDF can probe the structure-function relationship in diverse and complex materials including crystalline, disordered, amorphous, nanoscale and liquid systems. Carefully designed experiments allow exploring subtle changes in local environment of active elements and recognize hysteresis in behavior that can be linked to the limitations in battery performance. Operando measurements enable observations of elusive structural changes with remarkable precision providing confidence that observed changes are not a result of sample handling and/or contamination. Only through application of sensitive tools, aspects of structure, chemistry, electronic state and dynamics on appropriate scales, can lead to comprehensive understanding of materials performance needed for future design of improved systems. Here we present structure - function relationships that can be derived from PDF analysis in batteries.

**Keywords:** Electrochemistry, Energy, Materials Characterization, X-ray Diffraction

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

**Methodology Code:** X-ray Techniques
There has been growing interest in electrochemical reduction of carbon dioxide, a potent greenhouse gas and a contributor to global climate change. Given the fact that the carbon dioxide molecule is very stable, its electroreduction processes are characterized by large overpotentials. To optimize the hydrogenation-type electrocatalytic approach, we have utilized metallic centers (e.g. Pd, Pt or Ru) in a form of highly dispersed and reactive nanoparticles generated within a supramolecular network of distinct nitrogen or oxygen-coordination complexes. Among important issues are the mutual competition between hydrogen evolution and carbon dioxide reduction as well as specific interactions with metallic sites. We have also explored the ability of biofilms to form hydrogel aggregates of microorganisms attached to various surfaces including those of carbon electrode materials. Upon incorporation of various noble metal nanostructures, highly reactive and selective systems toward carbon dioxide reduction have been obtained. Another possibility to enhance electroreduction of carbon dioxide is to explore direct transformation of solar energy to chemical energy using transition metal oxide semiconductor materials. We show here that, by controlled combination of metal oxide semiconductors (titanium (IV) oxide and copper (I) oxide), we have been able to drive effectively photoelectrochemical reduction of carbon dioxide, primarily to methanol.

Keywords: Chemically Modified Electrodes, Electrochemistry, Fuels\Energy\Petrochemical, Nanotechnology
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Electrochemistry
Here, we report an analytical method based on in-situ mid-infrared (MIR) spectroscopic monitoring of the photochemical conversion of CO2 using solvated electrons, which are generated by illumination of hydrogen-terminated diamond waveguides via pulsed UV laser radiation. The advantage of this spectroscopic approach is that potential reduction products can be directly identified in solution via their vibrational signatures. In addition, diamond attenuated total reflection (ATR) crystal can be directly illuminated, thereby ejecting electrons into the adjacent RTIL. Ionic liquids such as 1-methyl-1-propylpiperidinium bis(trifluoromethylsulfonyl)imide (PMPipe/TFSI) are ideally suited for MIR measurements, as they do not show absorption features in the relevant spectral regime (1500-2500 cm⁻¹).

First results clearly indicate that at the given experimental conditions oxalate was formed as reduction product from the conversion of CO2. Further experiments are targeted towards the influence of trace water on the obtained reduction products, and the determination of the associated conversion rates. Furthermore, spectroelectrochemical studies at boron-doped diamond ATR waveguides are anticipated for investigating the electrochemically supported conversion of CO2.

Keywords: Energy, FTIR, Spectroscopy
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Molecular Spectroscopy
Egg shells were calcined at 1000°C for 4 hours to obtain calcium oxide (CaO) which was investigated as a heterogeneous catalyst for the transesterification of waste cooking oil. The catalyst was characterized by Fourier transform infrared (FTIR) spectroscopy and thermogravimetric analysis (TGA). Process parameters such as methanol-to-oil molar ratio, catalyst concentration, and reaction time on biodiesel yield were all evaluated and optimized. A maximum biodiesel conversion of 99.11% was obtained at 9:1 methanol to oil molar ratio, 4 wt.% catalyst loading, 2 hours of reaction time, and reaction temperature at 65°C. The percentage conversion was determined by 1H NMR. Additionally, the biodiesel synthesized was subjected to treatment methods including the hydrogenation of the double bonds in the FAME which are responsible for fuel gelling at low temperatures. The emission profile for various combinations of the biodiesel produced and the ultra-low sulfur petroleum diesel in a diesel-powered generator was characterized by gas chromatography coupled to mass spectrometry (GC-MS) and FTIR. The fuel mixture with 30% biodiesel blend was found to yield the lowest levels of carbon monoxide and methane.
Tier 3 gasoline standards call for very low sulfur measurements and these specifications may be put into effect in certain states in the United States starting in 2017. The new standards are meant to reduce emissions of passenger vehicles and trucks and lower the sulfur content of gasoline to reduce air pollution. The new requirement will only allow a maximum of 10 ppm sulfur in gasoline. ASTM D7220 “Standard Test Method for Sulfur in Automotive, Heating, and Jet Fuels by Monochromatic Energy Dispersive X-ray Fluorescence Spectrometry” can be used to verify that your gasoline meets the new EPA requirements. This study shows that current EDXRF Technology using matrix-matched empirical calibrations can be used for the EPA performance-based testing.
Traces of moisture found in petroleum products will cause corrosion on interior surfaces, potentially damaging pipelines during transportation. An innovative instrument and protocol has been developed to study this corrosion in the laboratory in a very short period of time. Until now, the method used for determining the corrosive properties of gasoline and distillate fuels in preparation for transport through a pipeline was developed by the National Association of Corrosion Engineers. The NACE TM0172 test method takes four hours to perform and requires 300mL of sample. While this has served the industry well for many years, there has been a strong interest for a method with faster turnaround, smaller sample size, and better repeatability and reliability.

Working closely with key refineries over the last few years, and with numerous trial and error experimental setups, a quicker, easier, and more reliable method has been developed. This ingenious laboratory experimental technique will save the industry significant time especially at pipeline transfer stations where a quick QC turnaround is necessary to determine if the product passes the corrosivity test.

Based on this idea, a new method, ASTM D7548 for Determination of Accelerated Iron Corrosion in Petroleum Products, has now been developed. This new ASTM method requires only 50mL of sample and takes less than a fourth of the time to complete the test. This poster will discuss in detail the development of this test technique, and will also show comparative data between the NACE test and ASTM D7548, the accelerated iron corrosion test.
The primary uses of oil in an internal-combustion engine are to decrease wear, friction, and heat of the moving components, to aid in sealing engine cylinders, and for contaminant removal. Engine oil is typically changed based on manufacturer recommendations, but these recommendations are unable to compensate for the wide range of engine operating conditions. There is currently no accurate in-situ technique to determine the optimal intervals to replace the oil. This results in preventable, early-onset engine failure from contaminated oil, or wasted resources from removing serviceable engine oil.

In this study, Fourier transform infrared spectroscopy (FTIR) was used to analyze SAE 15W-40 Diesel engine lubricating oil to determine if spectral absorption signature differences exist among the eight different levels of water contamination (0%, 0.1%, 0.2%, 0.5%, 1%, 2%, 5% and 10%), to determine the extent water can be detected in the spectral signatures, and to determine the rate at which an oil/water emulsion begins after initial free water contamination. This procedure is repeated once a week over five weeks then once every two weeks over ten weeks, and finally once every month. Preliminary indications are that water content in oil can be observed at a water absorption peak of 3400 cm\(^{-1}\) on day 1 at a 10% contamination level and by week 5 at a 0.1% level. Principal component analysis and partial least squares regression methods will be used to analyze the results and will be compared with other suitable alternative analytical techniques such as Terahertz Time-Domain Spectroscopy.

Keywords: Contamination, FTIR, Petrochemical, Trace Analysis
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Vibrational Spectroscopy
We investigated photocatalytic reaction of anatase-type titanium dioxide (IV) (TiO$_2$) against a compound which is used as an active pharmaceutical ingredient (API) using terahertz spectroscopy and Raman spectroscopy. TiO$_2$ is often used as a medical additive for a light-shielding coat around a tablet. It is known that anatase-type TiO$_2$ has photocatalytic reaction against an organic compound by exposure of UV light (below 400 nm) but usually used for pharmaceuticals due to its physico-chemical property. Although TiO$_2$ in a tablet coat will be basically separated from an API in the core tablet, an unsuitable coating process sometimes causes dissolution of a core tablet surface. It gives occasion of coexistence of TiO$_2$ and an API at the interface. This undesired process trouble may cause unexpected quality problem such as coloring of tablet surface. However, detail of this phenomenon have not been studied. In order to investigate the mechanism of photocatalytic reaction of TiO$_2$ in pharmaceuticals, we analyzed photon and molecular vibration of TiO$_2$ and APIs. Ciprofloxacin hydrochloride monohydrate (CPFX.HCl.H$_2$O) which is one of the important quinolone antibacterial reagents was selected for this study. The dried residue of TiO$_2$ suspension in CPFX aqueous solution was used. After UV exposure (254 nm) for 1 hour, the intensities of phonon vibrations (infrared and Raman active) of CPFX around the TiO$_2$ particles decreased significantly. Moreover, the Raman peaks in mid-wavenumbers region were getting broad. These observations suggest that amorphilization of CPFX.HCl.H$_2$O has occurred by photocatalytic reaction. We will discuss about photocatalytic reaction of TiO$_2$ against hydrates.

**Abstract Text**

**Keywords:** Infrared and Raman, Molecular Spectroscopy, Pharmaceutical, Vibrational Spectroscopy

**Application Code:** Pharmaceutical

**Methodology Code:** Vibrational Spectroscopy
We have developed a Continuous Wave Terahertz (CW-THz) Laser Spectrometer. The THz laser was realized on the principle of Difference Frequency Generation (DFG) of two infrared laser beams in a Gallium Phosphide (GaP) crystal. The spectrometer has the merits of wide frequency range tunability (0.5 – 6.0 THz), high resolution (< 10 MHz), high accuracy (a few MHz), wide dynamic range (>10^3 at the maximum), high long term stability (<0.3%), easy operation and almost no maintenance required. Using a superconducting Transition Edge Sensor (TES) bolometer cooled by a pulse tube refrigerator as a detector, the spectrometer became a non-stop system.

Our targets of such high accurate THz spectrometer is evaluation of organic crystals like as pharmaceuticals. We found absorption lines in some organic molecular crystals in middle molecular weight (400 – 2000 Da) could be resolved with a few GHz linewidth only at low temperature (~ 10 K). As a lot of absorption peaks existed adjacent in such molecules, no peaks could be observed due to wide linewidths at room temperature. For accurate evaluation of peak position and linewidth of these absorptions, MHz order accuracy was necessary. For example, THz absorption spectra of cyclodextrin hexahydrate (C[sub]36[/sub]H[sub]60[/sub]O[sub]30[/sub].6H[sub]2[/sub]O, MW=1080.94) sometimes used to improve the solubility of poorly water-soluble pharmaceuticals, purchased from four different company were obtained at 10 K. Comparing peak heights and linewidths with each other, apparent difference which seems to be affected by their crystalinity could be observed. Potential of high resolution and high accuracy THz spectroscopy will be discussed.
Morphology Directed Raman Spectroscopy (MDRS) is a diverse method that combines both white light microscopy and Raman spectroscopy for particle size/shape classification and chemical identification. The MDRS method has been previously shown to be successful in measuring API-specific particle size distributions within drug formulations. In this study, MDRS was used to investigate the feasibility of the method for conducting particle size analysis while simultaneously distinguishing between active pharmaceutical ingredient (API) particles dispersed within oil-based formulations. The chosen drug product for analysis in this work is a steroid/antibiotic combination ophthalmic ointment that contains tobramycin (0.3%) and dexamethasone (0.1%) as the active pharmaceutical ingredients (APIs). This project is of interest to the Food and Drug Administration due to the need to establish and to validate bioequivalence guidelines for ophthalmic ointments.

***Disclaimer***
This abstract reflects the views of the author and should not be construed to represent FDA’s views or policies.

Keywords: Microscopy, Particle Size and Distribution, Pharmaceutical, Raman Spectroscopy
Application Code: Pharmaceutical
Methodology Code: Microscopy
Giant Lipobeads as a Tool to Optimize the Number of Steps in Preparation of a New Smart Drug Delivery System

Our ultimate goal is to develop a new encapsulated medication regarding lipobeads as multipurpose containers. Giant lipobeads with temperature sensitive hydrogel core made of poly(N-isopropylacrylamide) (PNIPA) were synthesized using two methods: mixing separately prepared microgels and phospholipid vesicles of micrometer or nanometer size and polymerization within vesicles. Fluorescent microgels (5 – 150 um) were prepared by thermal or UV polymerization in different solvents and their structural difference was revealed microscopically. The stronger PNIPA hydrogels exhibited a more pronounced volume change in comparison to granular hydrogels. These findings will allow us to design drug delivery systems with different drug release mechanisms. Lipidic formulations prepared by injection of ethanol solution of lipids into hot water were compared with giant vesicles prepared by the method based on lipid film hydration. Different lipidic formulations (phospholipids with the order-disorder phase transition temperatures below and above room temperature, with or without cholesterol) were tested in terms of their interaction with the surface of microgel. The important finding was that besides lipid coating, one can notice many unfused vesicles adsorbed onto the surface of microgels when mixed with giant multilamellar vesicles. Both SEM imaging and confocal microscopy showed that the small unfused vesicles could be readily washed out from lipobead suspension by a low-speed centrifugation, so that microgel spheres were completely covered by homogeneous lipid bilayers. Giant lipobeads were also prepared by gentle hydration of lipid film with a hydrogel forming solution followed by UV polymerization. It was shown microscopically that hydration of a hybrid agarose/lipid film resulted in more regular lipobeads with unilamellar lipid bilayer around central hydrogel core. This work also validated that the time for scaled fabrication of lipobeads could be reduced from days to hours.

Keywords: Biomedical, Drugs, Pharmaceutical, Polymers & Plastics

Application Code: Pharmaceutical

Methodology Code: Microscopy
Morphology Directed Raman Spectroscopy (MDRS) is an emerging technology combining white light microscopy and Raman spectroscopy for ingredient-specific particle analysis. Most notably, the MDRS method has been used to evaluate both API (active pharmaceutical ingredient) and excipient particles dispersed within drug formulations. In this study, MDRS was used to evaluate the polymorphic forms of mometasone furoate to identify the polymorphic transformation of the anhydrous form to the monohydrate form within nasal spray formulations. Two separate protocols were designed and conducted over 5-week periods: an “in-use” study in which the nasal spray was stored on the bench top at room temperature, and an accelerated aging study in which the nasal spray was stored in an environmental chamber at 40 °C and 75% relative humidity. The samples were evaluated at one-week intervals using MDRS to identify and classify the mometasone furoate-containing particles based on morphology and spectral correlation within the 1675-1750 cm⁻¹ Raman spectral region.

***Disclaimer***
This abstract reflects the views of the author and should not be construed to represent FDA’s views or policies.

Keywords: Microscopy, Particle Size and Distribution, Pharmaceutical, Raman Spectroscopy
Application Code: Pharmaceutical
Methodology Code: Microscopy
The United States Pharmacopeia (USP) has recently introduced two new chapters (232 and 233) dealing with elemental impurities in pharmaceutical products. Permissible limits are given in Chapter 232 for a number of elements. These limits were revised in Feb–2016.

Chapter 233 defines two standard methods (ICP-AES and ICP-MS) but these do not have to be used provided an alternative method meets the validation requirements specified. There are two levels of validation depending on whether the method only indicates that the samples are above or below the limit (Limit Procedure) or gives a concentration (Quantitative Procedure). Both procedures were carried out for Cadmium, Lead, Arsenic and Mercury in an oral electrolyte formulation.

An Atomic Absorption Spectrometer with Zeeman Graphite Furnace and Autosampler were used for the Cadmium, Lead and Arsenic analysis. This system was chosen because both Zeeman and Deuterium background correction can be utilised for graphite furnace analysis. This provides the capability to perform accurate analysis with almost any matrix. With the simple addition of a hydride generating accessory the Atomic Absorption Spectrometer can easily perform the analysis of Mercury.

Keywords: Atomic Absorption, Pharmaceutical, Quality Control
Application Code: Pharmaceutical
Methodology Code: Atomic Spectroscopy/Elemental Analysis
Pharmaceutical - Vibrational, Raman, Microscopy, and Others

Advantages of On-Line React IR and Quick UHPLC Analysis in Synthetic Reaction Monitoring

Process Analytical Tools (PAT), such as React IR, are valuable tools that enable process understanding in synthetic reaction monitoring. These tools are critical to control the progression of the reaction, particularly if safety concerns exist. Grignard-based reactions can be highly exothermic, and, thus, requires that the Grignard reagent be dose-controlled and consumption be monitored in real time. Excess accumulation of the Grignard reagent in the reactor along with the highly exothermic nature of the reaction can result in a considerable safety risk. Further results on other synthetic reactions demonstrate the value of online React IR analysis for process understanding.

In the Grignard-based reaction, close monitoring of the Grignard precursor, starting material, and product during scale-up was successfully performed by using an online React IR method and quantitative UHPLC method (3.5 minutes). By monitoring specific regions of the IR spectra, the Grignard precursor (2-bromopropene) consumption and surrogate product formation was closely tracked (scan every 2 minutes). This tool enabled real time analysis to be delivered to the scale-up operators to ensure the team’s safety was not at risk. Offline UHPLC analysis was also critical to monitor impurity formation as the reaction progressed. The quantitative data obtained with the UHPLC analysis allowed for React IR quantitative model to be developed that could be implemented for further reactions. Results presented here for different synthetic reactions demonstrate the value in real-time conversion monitoring by ReactIR and quantitative analysis by UHPLC for both process optimization and control to ensure process safety.

Keywords: FTIR, On-line, Pharmaceutical, Process Monitoring
Application Code: Pharmaceutical
Methodology Code: Process Analytical Techniques
Determination of solid state forms of an active pharmaceutical ingredient (API), particularly in a complex drug product, is challenging and critical to drug development. A rapid and accurate analytical method for both qualitative and quantitative analysis of pharmaceutical solids would have a significant time and cost advantage over traditional methods such as XRPD and ssNMR. Vibrational spectroscopy, such as near infrared (NIR) and Raman can be used to quantify different solid state forms of API in drug product. Robust and accurate analysis can further be achieved by combining with chemometrics (e.g. Partial Least Squares Regression). In this study, a method for rapid and reliable quantitation of amorphous contents in real tablets using NIR and Raman was demonstrated. The calibration curve shows validated correlation coefficient of >0.99, in both NIR and Raman PLS models. The developed model was successfully used to predict the amorphicity in pharmaceutical tablets. The effects of composition of excipients, compression forces, temperature and humidity on solid forms in formulation were evaluated. The results obtained in this study provide valuable information and important guidance for formulation development.

Keywords: Chemometrics, Near Infrared, Quality Control, Raman Spectroscopy

Application Code: Pharmaceutical
Methodology Code: Chemometrics
### Abstract Text

CYP119, an orphan cytochrome P450 from the thermophilic archaea [i]Sulfolobus acidocaldarius[/i] is a good model system for the study of the intermediate states in the P450 catalytic cycle. Cytochromes P450 bind and cleave dioxygen to generate a potent intermediate (compound I) capable of hydroxylating inert hydrocarbon substrates. The compound I form of CYP119 was previously reported to be stable relative to other P450s, hence CYP119 is an attractive choice in employing chemically inert substrates to stabilize this rather fleeting intermediate [sup]1[/sup]. Stabilization of this form will possibly facilitate the structural characterization with resonance Raman (rR) spectroscopy [sup]2,3[/sup]. We report the expression and purification of the thermostable cytochrome P450 CYP119. The oxygenated complex of this enzyme was studied by resonance Raman spectroscopy, employing cryoradiolysis in an attempt to generate and trap fleeting intermediates, including compound I. In another approach, compound I was generated by reacting the ferric enzyme, bound to inert perfuorodecanoic acid (PFDA), with meta-Chloroperoxybenzoic acid (m-CPBA). Monitoring of the reaction by UV-visible spectroscopy provided evidence for the presence of compound I persisting from 3 to 45s. Efforts are being made to acquire the rR spectrum using conventional flow mixing devices. A possible application of acidophilic and thermally-stable P450s like CYP119 is utilizing them for practical industrial purpose e.g. the synthesis of organic compounds requiring hostile environments like extremes of pH or temperature.

### References

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### Keywords:  
Infrared and Raman, Raman Spectroscopy, Spectroscopy, UV-VIS Absorbance/Luminescence

### Application Code:  
Pharmaceutical

### Methodology Code:  
Vibrational Spectroscopy
In this study, Raman and Fourier transform infrared (FT-IR) spectroscopic techniques were used to characterize commercial forms of Ibuprofen in a variety of forms. This study evaluated gel pellets, liquids or syrups, and solid tablets of generic and innovator forms of Ibuprofen available at local pharmacy stores. Ibuprofen or \((\pm)\ 2-(p\text{-isobutylphenil propanoic acid, (CH}_3)_2CHCH_2C_6H_4CH_3CH_2CO_2H\) is well known as a non-steroidal anti-inflammatory, analgesic and antipyretic agent used worldwide due to its higher efficiency and tolerance, lower adverse effects and toxicity. To further study Ibuprofen structure we employed and optimize an extraction process of the active ingredient of solid tablets with chloroform concentrating in the measurement of the area of the infrared band corresponding to the carbonyl group around 1720 cm\(^{-1}\). The infrared (IR) spectra were collected with a Bomem FTLA2000 FT-IR bench using a PIKE MIRacle single bounce diamond ATR accessory to confirm the structure of the samples and the quality of the extracted product. From our results we observed that colored or non-soluble excipients contained in some tablets interfere in the observation of the products and extraction process, as well as the spectra collection. For gel pellets the effect seems to be the same regardless of the dye employed. The results collected applying Raman technique using a BioTools RamTestTM Chem Handheld Raman Identifier spectrometer which operates in the visible spectral region at 532 nm excitation confirm the results obtained in our IR spectra for isolated samples and allow us to further evaluate Ibuprofen presence in multiple solid and liquid commercial forms.
Detection of nucleic acids using electrochemical sensors has received much attention due to their significance for point of care (POC) disease diagnosis. Here, we report a universal electrochemical four-way junction (UE4J) sensor that consists of a universal DNA stem-loop (USL) probe attached to a gold electrode and two adaptor strands (m and f) that hybridize to the USL probe and nucleic acid analyte to form a 4J structure. The major advantage of this approach over conventional approaches is improved selectivity and potentially reduced cost due to the ability to use the same USL probe for detection of multiple targets. The sensor shows a linear range from 0.5 to 5 pM with a detection limit of 300 fM for miRNA122 using electrochemical impedance spectroscopy (EIS). In addition, the charge transfer resistance (R_{CT}) was monitored for an analyte containing a single base mismatch (SBM). Even when the SBM analyte was in five-fold excess, the R_{CT} remained at the background level, demonstrating the high selectivity of the UE4J sensor. Furthermore, we demonstrate that DNA with another sequence can be detected using the same USL probe by tailoring the adaptor strands to be complementary to the new target, which illustrates the universality of UE4J platform. The universality and high selectivity can make the UE4J sensor useful for POC diagnostic tests.

Keywords: Biosensors, Electrochemistry, Electrodes, Nucleic Acids
Application Code: Bioanalytical
Methodology Code: Electrochemistry
Two important pharmacophores: Acridine and thiophene were widely studied as antitumor, antiparasitic and antibacterial agents (2,3,6). We hypothesize that a conjugate comprised of two pharmacophores with different mechanisms of antiproliferative action can result in enhanced DNA damage (5,6). In this work we report the synthesis of one synthetic DNA intercalator 7ESTAC01 based on the acridine linked with thiophene conjugate. We recorded redox properties of 7ESTAC01 involved in adsorption controlled quasi-reversible. The acridine-thiophene derivative and DNA interaction was investigated using the DNA-biosensors prepared by immobilizing dsDNA onto the glassy carbon surface (GCE) (3,4) and gold electrode (GE) surface linked with Thiol. The thiol group functionalized dsDNA with 52% guanine bases are effectively immobilized on the surface of the GE through the specific Au-S covalent bond. The interaction of 7ESTAC01 and DNA was detected by electrochemical sensing of the oxidation of the DNA purine bases on the Glassy Carbon and Guanine on Gold electrode. The appearance of guanine, Ep=1.03 V, and the adenine, Ep=1.29 V peaks demonstrates that the damage by the radicals caused distortion of the double helix and exposure of the bases that can be oxidized. In the intercalation using ssDNA in solution, the decrease in the peak height of both bases, were estimated with interactions between compounds and ssDNA and showed slight oxidation signal only to Guanine. As evident from UV-Vis Spectroscopy, the binding constant of 7ESTAC01 was evaluated to Kb = 6,57 x 10^4 L mol^-1.

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Keywords: Biosensors, Drug Discovery, Electrochemistry, Electrodes
Application Code: Drug Discovery
Methodology Code: Electrochemistry
Ion Sensing Membranes (ISMs) function in either passive or active mode. At present, active mode ISMs contain spiropyrans or photoacid generators for modulation and control. However, drawbacks such as photodegradation of the photoactive compound due to the use of UV light for activation need to be circumvented in order to be applied for in vivo analysis. These drawbacks could be remedied by utilizing visible-light activatable metastable-state photoacids. Recently, our research group proposed ISMs using metastable-state photoacids that operate under acidic conditions. Thus, making it impractical for biologically relevant in vivo metal analysis. Herein, we propose to tailor the core structure of known merocyanine metastable-state photoacids to tune the pKa towards its use in ISMs. This is achieved by 1) introduction of a methylene dioxy group, electron donating, conjugated with the photoacid core and 2) substitution of geminal dimethyl groups for a spirocyclohexyl group. Novel metastable-state photoacids were characterized by 1H, 13C NMR spectroscopy and DART mass spectrometry. pKa characterization was performed using spectrophotometric and potentiometric methods. We expect that newly functionalized metastable-state photoacids will improve the robustness of ISMs in the physiological environment.

### Abstract Text

With increasing demand for portable point-of-care sensors, ion-selective electrodes (ISEs) have attracted increased attention, due to their portability, versatility and ability to be adapted to microfluidic, miniaturized systems while exhibiting high sensitivity and selectivity for ion detection in situ \(^1,2\). Nevertheless, these systems lack a condition free-method for analysis of various ions, and a new sensor needs to be used for each measurement \(^2\). Therefore, we present the clover sensor. This solid-contact, microfluidic ISE, does not require a pre-conditioning step (via addition of ion of interest directly into the cocktail), allows multiplexed analysis and pipette-free sampling for use in decentralized settings. The clover sensor is composed of three petals: one petal contains the reference membrane (ionic liquid and copolymer methyl methacrylate-co-decyl methacrylate) and the remaining petals are a combination of iodine/potassium sensing membranes and sodium/calcium sensing membranes. Monitoring imbalances of the aforementioned ions is crucial for prognosis of diseases such as goiter, hypokalemia, hyponatremia, and hypocalcemia, among others which are linked to a particular ionic deficiency. Preliminary experiments show promising results with near Nernstian response to K\(^+\), Na\(^+\), Ca\(^+\) and I\(^-\), with sub-micromolar limits of detection.

### References


### Keywords:

- Ion Selective Electrodes, Lab-on-a-Chip/Microfluidics, Sensors, Trace Analysis
- Biomedical
- Microfluidics/Lab-on-a-Chip
Ion-selective optodes (ISOs) allow sensitive and selective ion detection based on ionophores, as well as optical measurement of ionic species including alkaline metals, where no classical metal indicators are available. Among a variety of configurations, particulate ISOs are easily fabricated simply by adding an organic solution of necessary reagents into an aqueous solution in the presence of a surfactant. However, ISO particles fabricated by freehand pipetting are generally not stably dispersed in water, due to their micrometer-size and polydispersity.

In this research, inkjet printing technique was applied to fabricate nano-sized, monodisperse ISO particles targeting highly reproducible and selective detection of potassium ions (K\(^+\)). In contrast to freehand-generated ISO particles (334.2 nm, PDI: 0.240), inkjet printing allows to fabricate particles with reproducible and narrow size distribution (234.5 nm, PDI: 0.163) suitable for further processing with an office printer. This feature of inkjet-generated ISO particles allows mass production of ISO-based paper devices with an office inkjet printer.

The fabricated particulate ISOs were transferred to a paper-based analytical device for the purpose of simple and low-cost assay procedure. Fabricated ISO particles were deposited onto filter paper by means of a piezo-electrically actuated inkjet printer. Calibration curves based on the hue values calculated from RGB color values of the paper surface showed typical sigmoidal response to K\(^+\) shown in Fig.1. The recorded calibration curves showed theoretical response to K\(^+\) according to the formulation of ISO response previously reported. Importantly, the very small standard deviations (coefficient of variance: 0.11 % to 0.50 %) achieved owing to the high reproducibility of inkjet printing are expected to enable “calibration-free” (batch pre-calibrated) colorimetric assays on paper-based devices.
Currently, point of care testing (POCT) of electrolyte ions is in great demand in emergency medical care and health monitoring. Paper-based sensing platforms are expected to be suitable for POCT because of their low cost, portability, disposability and external power-free liquid transportation.

This work demonstrates the determination of alkali metal cations (e.g. Na\(^{+}\)) using ionophore-based ion-selective optodes (ISOs) adapted to microfluidic paper-based analytical devices (microPADs). The developed vertically assembled microPAD (vPAD) combines a pH buffering system and the ISO sensing membrane into a single user-friendly device. Capillary force-driven sample liquid transportation enables automatic pH adjustment prior to the optical detection by the film-based ISO.

A two-phase equilibrium cation exchange reaction between an organic polymeric optode phase and the aqueous sample phase has been employed. Conventional microPADs are open-channel; therefore, rapid sample evaporation is usually observed. Here, the whole device is laminated for prevention of sample evaporation to maintain the equilibrium state. Multiple functionalized paper layers were introduced for simplified device fabrication and for modular adaptation of target cation and detectable concentration range. Paper layers also serve as integrated pH-buffer system, eliminating the need of separate pH measurement or buffering of samples.

A typical sigmoidal response has been confirmed between 10\(^{-5}\) and 1 M of Na\(^{+}\) at pH 6.0 based on colorimetric hue measurements. As seen in the attached figure, calibration curves obtained from both water-diluted NaCl samples (black circles) and pH buffer-diluted NaCl samples without buffer-integrated layer (red crosses) showed good agreement. The developed vPAD showed tolerance to basic and acidic sample conditions (pH 8.0 and 5.0, respectively) as well as selectivity against other cations due to the ionophore used (DD16C5). The logarithmic selectivity coefficient for Na\(^{+}\) compared to K\(^{+}\) was found to be -2.1. Modulation of the integrated pH resulted in the shift of the concentration response range in a similar manner to classical film-based ISOs. The ISO behavior on the paper was compared with classical film-based ISOs, and several parameters including the overall equilibrium constant ([\(iK[/i]/[sub]exch[/sub]) were considered.

Keywords: Clinical Chemistry, Integrated Sensor Systems, Lab-on-a-Chip/Microfluidics, Sensors
Application Code: Biomedical
Methodology Code: Sensors
Coulometric Determination of an Ion Using Thin-Layer Electrolysis Cell for Ion Transfer at the Liquid Interface

An ion-sensor such as an ion-selective electrode has been the most standard analytical method for diagnostic of electrolyte imbalance in human body resulted by kidney trouble and so on. Most of analyzers adopt potentiometry with the ion-selective electrode and response to logarithmic concentration of a target ion and its precision is not enough to recognize difference between normal level and abnormal level of electrolyte concentration in blood (less than 1 orders). Amperometry for the ion transfer at the liquid-liquid interface has been reported as more precise determination than ion-selective electrode because of linear relationship between current and concentration of the target ion. Amperometry for the ion transfer can be realized in similar system to ion-selective electrode, i.e., organic solvent (plasticizer), ionophore, hydrophobic electrolyte and PVC membrane. Amperometric determination [1-3] or stripping voltammetry [4,5] based on the ion transfer has been demonstrated by utilizing a similar sensitive membrane. In the present work, we demonstrate coulometric determination of ion based on the ion transfer at the liquid-liquid interface by using a thin-layer electrolysis flow cell [6]. The coulometric determination indicates the linear dependence of current on the concentration of the target ion. Moreover, this method does not required calibration curve since the amount of the ion can be evaluated from coulomb number based on Faraday’s law.


Keywords: Electrochemistry, Quantitative, Sensors, Voltammetry
Application Code: Clinical/Toxicology
Methodology Code: Electrochemistry
**Session Title**  
Probing Ion Intercalation in Next-Generation Battery Interfaces Using Coupled Electrochemistry and In Situ Raman Spectroscopy

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**Abstract Text**

Despite their widespread commercial use, mechanistic studies of lithium-ion batteries (LIBs) are needed to improve their performance and understand next-generation materials with tailored properties.\cite{1,2} Our group has introduced well-defined electrodes of few layer graphene (FLG) as a potential next generation LIB anode materials with tailored electrochemical activity. Previous work in our group has demonstrated that Li$^{+}$ ions favorably intercalate into regions of graphene sheets that have been structurally modified.\cite{3} Directed interfacial modifications may allow for fabrication of FLG with the ability to outperform current graphite LIB anodes.

Here, we present the simultaneous use of in-situ Raman spectroscopy and scanning electrochemical microscopy (SECM) for following structural changes experienced by patterned FLG during intercalation of alkaline ions. Use of an inverse confocal Raman microscope enables detection of sample behavior unimpeded by the ultramicroelectrode of the SECM, and minimizes interferences from solvent scattering. By pairing Raman spectroscopy and SECM, we gained a better understanding of how ion intercalation propagates disorder in FLG through spectral data, while probing the interfacial reactivity of the material with electrochemistry. The combined techniques also allow for a more comprehensive study of how formation of the secondary electrolyte interface changes the surface of the modified FLG and its reactivity.


**Keywords:** Electrochemistry, Energy, Raman Spectroscopy

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

**Methodology Code:** Electrochemistry
Non-aqueous redox flow batteries (NRFBs), promise high energy densities due to wide potential windows and a large variety of highly soluble species for efficient grid-scale energy storage.¹ To maintain high conductivity, NRFBs require porous separators that are easily solvated, yet these cannot fully prevent crossover of small molecules.² Macromolecular redox designs for NRFBs have been of recent interest in mitigating this parasitic crossover to prevent capacity fades. These size-exclusion materials, typically polymers³-⁴, are designed for use with porous membranes to maintain high conductivity.

Emphasizing the size-selective hypothesis in NRFBs, we have recently introduced redox active spherical particles in the submicron range made of cross-linked polymer chains that offer higher control over material geometry and size. From crossover studies, these particles show > 99% retention against nanoporous separators. Electrochemical studies of these in dispersion show that they exhibit both surface-confined and diffusional signatures. Bulk electrolysis experiments show that they can reversibly charge and discharge in dispersion without creating a passivating layer at the electrode. Thus, the adsorbed films formed at electrodes successfully mediate charge transfer to the bulk dispersion without degradation. Our study aims to elucidate on the mechanism of charge transfer that allows for the bulk redox activity in these particles. To do this, micro- and nano-electrodes are used to address individual particles by diffusional collisions. These collisions can elucidate on the mode of charging when particles come into electrode contact. In sum, redox active polymer particles demonstrate an interesting interplay of charge hopping from film to the bulk when dispersed that we are exploring via bulk and individual particle methods.


Keywords: Electrochemistry, Energy, Materials Characterization, Voltammetry
Application Code: Material Science
Methodology Code: Electrochemistry
Investigation of Photoanodic Water Oxidation Surface Species on Hematite Using SI-SECM

Hematite is a promising photoanode for solar driven water splitting because of its narrow band gap, chemical stability, and elemental abundance. However, high overpotentials are required to overcome sluggish water oxidation kinetics which curtails its efficiency and prevents practical application. To offer insight on the best strategies for improving hematite anodes, we use Surface Interrogation Scanning Electrochemical Microscopy (SI-SECM) to probe the reactive oxygen species (ROS) that are responsible for hematite’s slow kinetics. This technique uses an ultramicroelectrode to electrochemically generate a titrant from solution which reacts with ROS and generates a transient feedback signal as species are depleted from the surface. SI-SECM is unique in its ability to directly quantify local coverage and reactivity of surface species across the broad range of spatial and temporal scales relevant to lifetimes of adsorbed species during reaction.

Here, we demonstrate its use for simultaneously observing the decay of two populations of photogenerated ROS on thin films of hematite.

Satisfactory fitting of the SI-SECM signal obtained on hematite photoanodes required the existence of two populations of ROS reacting with different rate constants. We observed that while the surface coverage of both species increased as a function of applied bias, the rate constants did not change appreciably, showing that the identity of the species doesn’t change with increasing activation. The characteristic lifetimes of ROS were studied by varying the time spend undisturbed before titration. Kinetic analysis of these results showed that the decay of ROS happens through a second order process. Using pulsed substrate generation/tip collection mode, we detected hydrogen peroxide as a product of this decay.

Ref

Keywords: Electrochemistry, Electrode Surfaces, Quantitative, Semiconductor
Application Code: General Interest
Methodology Code: Electrochemistry
Nano- or micro-structured photocatalysts are attractive materials for solar fuel generation because of their high catalytically active surface areas. Recently, cathodic corrosion was introduced as a facile electrochemical method to fabricate nano- or micro-scale crystalline particles from bulk materials.[1] Here, we used the substrate generation/tip collection (SG/TC) mode of scanning electrochemical microscopy (SECM) as a screening tool to characterize the performance of WO$_3$ microparticles synthesized via cathodic corrosion.[2] We electrochemically collected oxygen produced by individual illuminated particles to quantify the average photocatalytic activity of particles. Oxygen collection experiments done with SG/TC SECM measured oxygen evolution photocurrents of 10 pA per particle, equivalent to a current density of 0.5 mA/cm$^2$. This value is comparable to other reported nanostructured WO$_3$ photocatalysts, and our particles have the additional advantage of having a much more facile fabrication process. Additionally, simulations using COMSOL Multiphysics 4.4 quantified the rate of O$_2$ production per particle as 104.3 ± 7.63 µmol s$^{-1}$ m$^{-2}$ under illumination. This combined process of producing photocatalyst particles by cathodic corrosion and screening the photoelectrochemical activity of individual particles with SECM demonstrates a versatile workflow to assess the intrinsic performance of nanomaterials for photocatalysis.


Keywords: Electrochemistry, Fuels\Energy\Petrochemical, Materials Characterization, Microelectrode
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Electrochemistry
We have introduced a new concept for redox flow batteries based on size-selectivity. [1] This system requires macromolecular designs with built-in redox functionality and uses simple porous separators. Efficient 3D-charge propagation must occur within the macromolecular structures in order to ensure fast charge transfer kinetics, reversible energy storage, and adequate cyclability [2]. To understand the factors that affect macromolecular redox active polymers and redox mediation, it is desirable to isolate their behavior by probing discrete units such as films and particles.

We investigated charge transfer and transport within particles and thin films made of redox active polymers. Direct probing of redox processes using scanning electrochemical microscopy [3] with nano-electrodes was used to effectively isolate and analyze kinetic parameters of single polymer particles (80-830 nm in diameter) and polymeric films based on ferrocene and viologen moieties. Electrochemical measurements, including cyclic voltammetry and bulk electrolysis, were used to evaluate the performance of particles of varied sizes and composition within various solvents. Methodologies based on micro- and nano-electrode measurements and COMSOL simulations were utilized to evaluate the propagation of charge. We investigated the dependence of charge/discharge curves on both morphological parameters such as polymer film thickness/polymer molecular weight/particle size/shape, as well as chemical parameters such as supporting electrolyte and solvent type.


Keywords: Electrochemistry, Energy, Nanotechnology, Polymers & Plastics
Application Code: Polymers and Plastics
Methodology Code: Electrochemistry
New investigations of ion transfer reactions at energy storage materials are enabled by Hg-based Scanning Electrochemical Microscopy (SECM) imaging modes.\[sup\]1,2[/sup] Here, we discuss quantitative approaches for the positioning and operation of these probes with battery interfaces in mind. Because the operational conditions for many battery electrode materials are harsh and can easily decompose redox mediators, traditional SECM probe positioning methods are challenging to implement. Hg-based probes are uniquely capable of circumventing this issue by performing amalgamation reactions with metal cations. Since the product is transferred into the Hg phase, unwanted side reactions are not an issue. However, potentiostatic amalgamation eventually leads to saturation of the amalgam accompanied by irreversible loss of Hg. In order to obtain negative feedback positioning control without risking damage to the SECM probe, we implemented cyclic voltammetry probe approach surfaces (CV-PASs), consisting of CVs performed between incremental motor movements. The amalgamation current, peak stripping current, and integrated stripping charge extracted from a shared CV-PAS give three distinct probe approach curves (CV-PACs), any of which can be used to determine the tip-substrate gap to within 1% of the probe radius. We establish a new protocol for fitting any CV-PAC and demonstrate its validity with experimental results for sodium and potassium ions in propylene carbonate. Using Comsol simulations, we also present limiting conditions for obtaining and fitting CV-PAS data. This is the first report of alkali ion CV-PASs as well as the first time CV-PAC data of any kind has been used to precisely and reproducibly position an SECM probe.


**Keywords:** Electrochemistry, Energy, Microelectrode, Stripping Analysis

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

**Methodology Code:** Electrochemistry
Ion-Transfer Voltammetry in Perfluorinated Matrices: Detection of Perfluorooctanesulfonate and Perfluorooctanoate in Clinical Samples

Perfluorinated compounds have been synthetically produced since the 1950s but have only recently been linked to diseases such as chronic kidney disease. Two of the most prevalent of these compounds are perfluorooctanesulfonic acid (PFOS) and perfluorooctanoic acid (PFOA). The current detection technique for PFOS and PFOA is solid-phase extraction with liquid chromatography/tandem mass spectrometry. This technique has a 1.4 and 1.7 ng/L limit of detection for PFOS and PFOA but requires extensive sample preparation and expensive instrumentation. Therefore, it is the goal of this project to develop a more affordable and time-efficient technique that can detect PFOS and PFOA in small volume clinical samples by taking advantage of the fluorophilic nature of PFOS and PFOA. For the first time a fluorophilic liquid membrane will be developed for ion-transfer stripping voltammetry using a solid-contact electrode (gold electrode, conductive polymer, and fluorophilic membrane). To achieve this goal, solid-contact electrodes with a fluorous membrane for ion-transfer stripping voltammetry are being developed. Using this technique, ultralow detection limits are made possible by preconcentration of the analyte in the fluorous membrane at an applied voltage prior to detection of the large current when the analyte is quickly released back into the sample (i.e., stripping voltammetry). This process takes advantage of the high selectivity of fluorous ion-selective electrodes, while allowing for lower detection limits. Also, it allows for the detection of non-redox active species, which is not possible for conventional stripping techniques.

Keywords: Electrochemistry, Stripping Analysis, Surfactants, Voltammetry
Application Code: Clinical/Toxicology
Methodology Code: Electrochemistry
Good electrode-to-electrode reproducibility is a necessity for calibration-free electrochemical sensors. Previously, our group introduced cobalt(II)/cobalt(III) redox buffers as an inner reference to improve the electrode-to-electrode reproducibility, making calibration-free measurements possible [1]. However, due to its low lipophilicity, leaching of the redox buffer from the ion-selective membrane into the aqueous sample could not be prevented, resulting in potential drifts. In this work, we develop a new class of polymeric redox buffers by attaching cobalt(II)/cobalt(III) redox couples to hydrophobic polymers through covalent bonds and use them for the fabrication of solid-contact ion-selective electrodes that exhibit exceptional long-term stability by avoiding leaching of the redox buffers from the ion-selective membrane into the aqueous sample. Infrared spectroscopy, differential scanning calorimetry and cyclic voltammetry were used to characterize the polymeric redox buffers, showing that cobalt couples were attached to the polymer without affecting the glass transition temperature and that the redox reaction between cobalt(II) and cobalt(III) species is reversible.


Keywords: Electrochemistry, Electrodes, Sensors
Application Code: General Interest
Methodology Code: Electrochemistry
Redox buffers are components containing both oxidized and reduced species of a redox couple. In analogy to a pH buffer that exhibits an adjustable pH value, the potential of a redox buffer is stable, reproducible, and can be controlled by the ratio of its oxidized and reduced species. Herein, a porous carbon-based all-solid-state redox buffer is developed that can be used as an essential building block in electrochemical systems where controllable and stable interfacial potentials are needed. Colloid-imprinted mesoporous (CIM) carbon is surface-functionalized with Co(II) and Co(III) complexes, and a redox buffer can be prepared by mixing the two functionalized CIM carbon material. By varying the ratio of Co(II) to Co(III) attached to CIM carbon, the potential of the redox buffer can be controlled. Furthermore, the application of this all-solid-state redox buffer in potentiometric ion sensing is discussed.

Keywords: Bioanalytical, Material Science, Nanotechnology, Sensors
Application Code: Material Science
Methodology Code: Sensors
Fluorous-phase ion-selective electrodes (ISEs) exhibit large improvements in sensing range compared to conventional polymeric matrix-based ISEs. Due to the extremely low polarity of their sensing membranes, both interfering ions and counter ions experience extremely low solvation, thus substantially expanding the sensing range. Stronger binding between target ion and ionophore due to fluorous-phase’s non-coordinating nature also contributes to improved selectivity. Previously developed fluorous-phase ISEs based on two fluorophilic ionophores have sensing ranges of pH 1.5 to 6.5 and 6.0 to 13.0, respectively. The objective of this research is to develop a new fluorous-phase ISE with a neutral pH centered sensing range, suitable for physiological pH sensing. To achieve this, a new fluorophilic ionophore tris(perfluoro(octyl)butyl)amine was synthesized and a new sensing electrode platform was developed. With superior electrode sealing, the new platform offers remarkably enhanced long-term sensor stability. Fluorous-phase ISEs based on this new ionophore and sensing platform exhibit excellent sensing range from pH 2.2 to pH 11.6, which is by far the widest range in pH ISEs and covers most of physiologically relevant pH ranges. Furthermore, excellent selectivities against common interfering ions such as K\(^+\), Na\(^+\) and Ca\(^{2+}\) are determined (Selectivity Coefficients: \(\log K_{H,K}^{\text{pot}} = -11.6\); \(\log K_{H,Na}^{\text{pot}} = -12.4\); \(\log K_{H,\text{Ca}}^{\text{pot}} < -10.2\)). With the unique character of fluorous-phase, this significant advancement in fluorous-phase pH ISEs offers an excellent candidate to combat the challenge of biofouling, as well as setting the ground for biocompatible and miniaturized sensor developments.

Keywords: Bioanalytical, Biosensors, Clinical Chemistry, Ion Selective Electrodes

Application Code: Bioanalytical

Methodology Code: Sensors
Microbiologists in the research and biotech industry still primarily depend on bacterial culture plates to identify bacterial species. There are existing bacterial identification methods that reduce the time to acquire results from days to hours with improved sensitivity such as genetic identification using polymerase chain reaction (PCR) and other molecular methods that detect DNA hybridization. However, these technologies require costly equipment that do not scale and cannot be automated for industrial applications.

Our proposed technology provides an alternative low-cost, real-time bacterial detection platform that can potentially provide in-line bacterial process monitoring in pharma and biotech manufacturing. The technology uses low cost electrochemical sensors for detecting quorum sensing molecules excreted from bacteria. Our lab has previously used electrochemical sensors to detect one of the quorum sensing molecules, named pyocyanin, which is produced uniquely by Pseudomonas aeruginosa. The concentration of the pyocyanin was correlated to be proportional to the bacterial density.

In this work we explore how the sensitivity of the electrochemical sensors can be improved by selectively concentrating the target molecules using dialysis in an online sampling system. We expect that this approach will be valuable for monitoring and early detection of contamination in bioreactor systems.

**Keywords:** Biosensors, Electrochemistry, On-line, Process Analytical Chemistry

**Application Code:** Process Analytical Chemistry

**Methodology Code:** Electrochemistry
We have developed carbon based solid state pH ultra-micro sensor to study bacterial metabolic activity. These fast responsive sensors showed a nernstian slope of 58 ± 2 at 37°C in artificial saliva solution. Due to having high carbon content these sensors are capable of amperometric measurement. Using Scanning Electrochemical Microscope (SECM) with these newly developed probes we have studied the change in local pH, submicron above the biofilm. In addition, we have also synthesized a hydrogel to immobilize bacteria to make a stable biofilm for real time monitoring the metabolites with SECM probe. This hydrogel was also tested for the bacterial viability for various bacterial species and this synthetic hydrogel shows biocompatibility. This was further confirmed by using a confocal microscopy with gfp tagged E. coli-hydrogel model system. The recent development of the work will be present in the conference.
Measuring biomarker levels at early stages of cancer and neural diseases would decrease fatality rates and allow successful treatment outcomes. However, the ultralow levels of most biomarkers that are indicative of disease conditions compels the need for sensitive diagnostic methods that additionally possess simplicity and selectivity. Moreover, the molecular size of a biomarker influences the detection limit and sensitivity of diagnostic methods. Use of many nanotechnology strategies with optical and electrochemical detection methods has allowed detection of large proteins, receptors, antibodies, DNA, and RNA biomarkers at clinically relevant pM to aM concentrations in body fluids. Compared to the detection of large biomolecules, small molecule markers pose a huge challenge to designing assay and detection strategies that can achieve clinically relevant parts-per-billion (ppb) detection. In this study, we demonstrate for the first time flow injection amperometric detection of formaldehyde in a urine matrix with specificity via formaldehyde dehydrogenase immobilized pyrenyl-nanostructure electrodes offering 6 ppb detection limit and a dynamic range of 10 ppb to 10 ppm. The biosensor was constructed by covalent immobilization of NAD+ dependent formaldehyde dehydrogenase onto 1-pyrenebutyric acid units pi-pi stacked with multiwalled carbon nanotubes on the surface of gold screen printed electrodes. Such sensitive nano-biosensor strategies are useful for early non-invasive diagnosis of cancer and other deadly neural diseases based on ultralow level detection of small molecules as markers.

Keywords: Biosensors, Electrochemistry, Electrodes, Nanotechnology

Application Code: Clinical/Toxicology

Methodology Code: Electrochemistry
Scanning Electrochemical Microscopy (SECM) is used to study the local chemical environment above live bacterial biofilms such as S. gordonii (Sg), S. mutans (Sm) etc. In this study, we report the development of a Pt decorated carbon nanotubes (CNTs) based dual SECM probe to detect low micro-molar range hydrogen peroxide (H2O2) produced by the live bacterial biofilm. This sensor showed sensitivity 2.2 ± 5 mA cm-2 mM-1 with low detection limit of 0.5 µM. Our results indicate that the Sg produced 65-70 µM H2O2 within 30 min in presence of 1 mM glucose in artificial saliva solution (pH 7.2) at 37°C. In addition, we have also developed a unique solid state carbon based potentiometric pH sensor and used as a SECM probe to map the pH change at high spatial resolution above the Sg biofilm. The sensor showed Nernstian response with slope of 58±4 and very fast response time of 5s. The pH mapping above the biofilm showed that pH above the biofilm dropped by one pH unit within 30 min only in 6 pH artificial saliva with 30 mM sucrose at 37°C. Recent developments about the real-time metabolic exchange between two bacterial species (Sg and Sm) in terms of peroxide and pH will be presented in the conference.

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**Keywords:** Bioanalytical, Biosensors, Electrochemistry, Electrodes

**Application Code:** Bioanalytical

**Methodology Code:** Electrochemistry
Dopamine is an important neurotransmitter in the central nervous system because it is associated with various behavior outputs including reward, motivation, and motor function. Dysfunction of dopamine signaling can lead to neurodegenerative diseases such as Parkinson’s disease, Alzheimer, and schizophrenia. Therefore, understanding the mechanism of dopamine signaling is significant. Drosophila melanogaster, also known as fruit fly, has been emerged as an excellent animal model in many research area such as neuroscience, toxicology, and genetics because of its homology to mammals and easy of genetic manipulations. Our group has developed a method using fast-scan cyclic voltammetry (FSCV) at carbon fiber microelectrodes to detect optogenetically stimulated neurotransmitters release and uptake in Drosophila larvae. In this study, we measured endogenous dopamine release in adult Drosophila brain, upon the optogenetic stimulation. In addition, stimulating parameters such as, recovery time, number of pulses, and stimulating width were optimized. This study will allow us to characterize dopamine regulation under the different pharmacological conditions or in genetically modified adult Drosophila.

**Keywords:** Electrochemistry, Neurochemistry, Optogenetics, Voltammetry

**Application Code:** Neurochemistry

**Methodology Code:** Electrochemistry
Voltammetric Determination of Diffusion and Partition Coefficients in Plasticized Polymer Membranes

Abstract Text
The diffusion-controlled transport of ions and molecules through polymer membranes utilized in chemical and biosensors is often the key factor determining the response of these sensors. Here a simple voltammetric method for the quick and simple determination of diffusion coefficients in resistive soft polymer membranes using a planar electrochemical cell (PEC) with carbon fiber microelectrode as working electrode is described. The feasibility of the voltammetric method has been demonstrated by measuring the diffusion coefficients of ferrocene derivatives in highly resistive aqueous solutions, organic solvents, plasticizers, soft hydrogel films, and plasticized PVC membranes. The measured diffusion coefficients are in agreement with theoretical models and previously reported values. The voltammetric method has been used to assess the diffusion coefficients in the membrane coating of a voltammetric propofol sensor. The diffusion coefficients of propofol (analyte) and p-acetamido phenol (interferent) in 2-nitrophenyl octyl ether and bis(2-ethylhexyl) sebacate plasticized PVC membranes with different plasticizer/polymer ratios are reported as well as the diffusion coefficient of tris(1,10-phenanthroline) Ru(II) chloride in soft polyvinyl alcohol hydrogels.

Keywords: Biomedical, Biosensors, Electrochemistry, Sensors
Application Code: Biomedical
Methodology Code: Sensors
For years now vibrational spectroscopy has been an integral part of the plethora of methods for analyzing pharmaceutical samples. The most exciting applications of vibrational spectroscopy may be found in its combination with chemometrics, specifically in process analytical technology (PAT) or chemical imaging. In this paper we present several cases from industrial practice.

Raman chemical mapping of Xanax/Alprazolam tablets illustrates the use of principal component analysis (PCA) in identifying weak Raman signal of the active pharmaceutical ingredient (API). Univariate imaging of the above tablets produced noisy images with unclear spectral response behind hot pixels and hence ambiguous assignment of the images. The PCA clearly identified the API signal and the imaging via PC scores was shown to be much more effective for identifying the API Raman signal and locating API domains.

Online monitoring of API concentration during the blending process is one of more popular PAT applications of NIR spectroscopy. In this study, an NIR probe is set in the feed frame of the tablet press and NIR signal is obtained immediately before the powder entering the press. Large variations in API concentrations are mimicked in order to determine the sensitivity of the NIR response of the given API. PCA is again used to aid identifying the API signal in the blend and determine relative API concentration throughout a lengthy run of the press.

Finally, the performance of transmission Raman instrument is compared with IR-based techniques, diffuse reflectance IR FT (DRIFT) and ATR, for quantitative determination of API polymorphs in powder API mixtures. While successful in identifying agglomeration of the impurities, transmission Raman spectroscopy was less effective in multivariate calibration of those impurities than both DRIFT and ATR methods. DRIFT spectroscopy proved most accurate for quantifying the impurities in the concentration span of 0 to 2% wt/wt.

Keywords: Chemometrics, Infrared and Raman, Pharmaceutical, Raman Spectroscopy
We have studied chemometrics methods for extracting the paraffin component from paraffin-embedded oral cancer tissue Raman spectra. Three kinds of multivariate analysis (MVA) methods; Independent Component Analysis (ICA), Partial Least Squares (PLS) and Independent Component - Partial Least Square (IC-PLS) were tried for the above purpose. The estimated paraffin components were used for removing the contribution of paraffin from the tissue spectra. The efficiency of paraffin removal and the ability to retain the tissue information of these three methods were compared. We found that ICA, PLS and IC-PLS were able to remove the paraffin component from the spectra at almost the same level while Principal Component Analysis (PCA) was incapable. In terms of retaining cancer tissue spectral integrity, effects of PLS and IC-PLS on the non-paraffin region were significantly less than ICA where cancer tissue spectral areas were deteriorated. By using the paraffin-removed spectra Raman images of oral cancer tissue were developed, and the images were compared with Hematoxylin and Eosin (H&E) stained tissues for verification. This study has demonstrated the capability of Raman spectroscopy together with multivariate analysis methods as a diagnostic tool for the paraffin-embedded tissue section.
Polymorph detection, identification, and quantitation in crystalline materials is of great importance in the pharmaceutical industry. Low frequency Raman spectroscopy is of particular interest for the study of pharmaceuticals because of the recent availability of moderately priced, high performance Raman systems capable of measurements in the range of 200-10 cm\(^{-1}\). The large aromatic molecules typical of many APIs have characteristic Raman bands below 200 cm\(^{-1}\) deriving from both fundamental molecular vibrations as well as external lattice vibrations. The large aromatic molecules in solid crystalline state often exhibit bands between 200-100 cm\(^{-1}\) which derive from fundamental molecular vibrations while bands below ca. 50 cm\(^{-1}\) derive from the lattice modes. The lattice vibrations of molecular crystals more directly probe the intermolecular interactions of molecules in the solid state. We demonstrate the low frequency Raman characteristics of amorphous and crystalline APIs, provide a general survey of selected APIs and demonstrate the sensitivity to crystalline forms.

**Keywords:** Pharmaceutical, Raman Spectroscopy

**Application Code:** Pharmaceutical

**Methodology Code:** Vibrational Spectroscopy
In order to commercialize clinical sensors for optical detection of human disease, to combat terroristic threats associated with improvised explosive devices (IEDs) and chemical warfare agents, ChemImage has been investing in the development of smarter sensors that provide end-users real-time, high confidence, autonomous awareness of their environment. Chemical Imaging has proven to be a beneficial method as the foundation for these sensor platforms, specifically wide area Chemical Imaging sensors that utilize shortwave infrared (SWIR) absorption and/or Raman scattering spectroscopic techniques. The current generation Chemical Imaging sensors found their first application, in previous generation instruments, in the field of pharmaceutical analysis, including ingredient-specific particle sizing (ISPS).

Competing sensors have shortcomings that make their implementation into field use difficult, including size, weight, and power (SWaP) restrictions, diminished area search rate and eye/tissue-safety concerns. Current generation SWIR and Raman Chemical Imaging sensors overcome these shortcomings by providing operators with small size, lightweight (wearable and handheld) and low power consuming configurations. These sensors also provide high area search rates, including in many cases real-time operation, while minimizing eye and tissue-radiation safety concerns. Specific end use applications include intraoperative imaging and predictive heart failure detection, as well as explosives, chemical warfare agents, and narcotics detection. Raman Chemical Imaging sensors are configured in both standoff and bench top configurations, with an emphasis on improving the area search rate and developing eye-safe standoff sensors.

This presentation will provide an overview of current and emerging strategies for automated detection of disease and hazardous materials using smart Chemical Imaging sensors. Use scenarios and detection results for these sensors will be discussed.

Keywords: Detection, Imaging, Infrared and Raman, Sensors
Application Code: Homeland Security/Forensics
Methodology Code: Sensors
The Coblentz Society - Williams-Wright Award

**Raman, Mid-Infrared and Near-Infrared Spectroscopy with Handheld Instruments: Instrumentation, Applications and Future Aspects**

Fourty years ago Raman and FT-IR spectrometers almost occupied a separate room and NIR spectrometers were just about to quit the phase as add-ons to UV-VIS or IR spectrometers and appear as stand-alone instruments. The following four decades were characterized by a multiplicity of exciting hard- and software developments for vibrational spectroscopy but apart from opening the lab to the process by the introduction of light-fiber optics, special probes and chemometric evaluation routines the techniques remained a domain for scientists. In contrast, the recent development of miniaturized, handheld instruments has not only led to a further extension of the range of applications by on-site and in-the-field measurements but also shows promise that these instruments may in the future be used by non-traditional user environments.

The reduction in size, however, must not lead to compromises in measurement performance and precision and the handheld instrumentation will only have a real impact on quality and process control if Raman, IR and NIR spectra of comparable quality to laboratory spectrometers can be obtained.

The presentation will provide an overview on the building principles and performance parameters of state-of-the-art handheld systems, discuss the pros and cons of the different techniques and will highlight the advantages of on-site measurements by means of selected application examples.

Finally, the transfer of spectra that have been measured on a laboratory FT-NIR spectrometer to the format of a handheld instrument by measuring only a few samples with both spectrometer types will be shortly demonstrated. Thus, despite the extreme differences in spectral range and resolution, data sets which have been collected and calibrations which have been developed thereof, respectively, over a long period on a laboratory instrument can be conveniently transferred to a handheld system without the requirement for elaborate rescanning and recalibration of spectra.

**Keywords:** Food Identification, Infrared and Raman, Near Infrared, Portable Instruments

**Application Code:** General Interest

**Methodology Code:** Portable Instruments
Microfluidics enables rapid, low-volume sample analysis. We have investigated use of microfluidic systems for monitoring neurotransmitter and hormone dynamics. The first project discussed is "chip in a body" and is development of a microfluidic sampling probe that can be inserted into the brain. The probes are 50 um x 50 um and greatly improve spatial resolution relative to microdialysis and other sampling techniques. The sampling solution is coupled to microscale methods such as CE and MS to measure neurotransmitters over time. The second project is "body on a chip". In this case, we place multiple cell types on one chip with analytical monitoring components to detect how the cells affect each other. In particular we demonstrate the effect of adipocytes on insulin secretion from islets. These examples represent advances in use of microfluidics for chemical monitoring of biological systems.
Nitric oxide (NO) has many physiologically important properties, including serving as a potent antithrombotic, antimicrobial and anti-inflammatory agent, as well as facilitating angiogenesis and vasodilation. These properties make NO release/generation potentially useful for developing improved medical devices, including advanced thromboresistant/bactericidal intravascular (IV)/urinary catheters, in vivo chemical sensors, wound healing patches, and insulin infusion cannula, as well as methods to create gas phase NO for inhalation therapy. In this presentation, recent efforts to develop both chemical and novel electrochemical approaches for generation of NO for use in the development of such biomedical devices/systems will be described. The most recent and promising chemical-based method involves use of S-nitroso-N-acetylpenicillamine (SNAP) as an NO donor species impregnated into various plastic medical tubing/films. Devices prepared with SNAP-doped polymers exhibit long-term NO release (up to 1 month), reduced clotting in vivo and significantly less microbial biofilm formation on their surfaces when tested both in vitro and in vivo. The electrochemical approach is based on the electro-reduction of inorganic nitrite anions using Cu(II)-ligand complexes as mediators that mimic the active sites of nitrite reductase enzyme. The temporal pattern of electrochemical NO generation can be precisely modulated/controlled by the magnitude of different applied potentials or currents. This new electrochemical NO generation chemistry can be readily incorporated within one lumen of multi-lumen intravascular catheters to reduce clotting and infection, and improve the accuracy of intravascular PO2 sensors. Further, it will shown that the new electrochemical NO delivery method can also be adapted to develop a novel NO inhalation (INO) system that can potentially replace the costly NO tanks used currently for INO in hospitals to treat pulmonary hypertension.
Amino acids and neuropeptides play critical roles as cell to cell signaling molecules between neurons and other cells. A detailed understanding of their formation, presence and structure is important for understanding brain function in health and disease. Using several small volume separation and mass spectrometry-based approaches, we have characterized both free d-amino acids and d-amino acid containing neuropeptides in the nervous systems of several animals. We combine chromatography and mass spectrometry-based measurements to identify endogenous neuropeptides that have D-amino acids, an unusual post-translation modification that can have a profound impact on receptor signaling. We have used these methods to discover novel D-amino acid-containing neuropeptides that function in the central nervous system of *Aplysia californica*, and have characterized the function of several of these neuropeptides in terms of receptor signaling and animal physiology. In the same animal, we use capillary electrophoresis to measure the amino acid transmitters within selected cells. We have characterized the conversion of free amino acids from their L- to D-form. In the case of D-aspartate, the D-form fulfills the criteria for a classical transmitter, demonstrating the importance of fully characterizing the chirality of cell-cell signaling molecules. We are currently studying other D-amino acids including D-glutamate found within *Aplysia*.

**Keywords:** Capillary Electrophoresis, Mass Spectrometry, Neurochemistry

**Application Code:** Neurochemistry

**Methodology Code:** Mass Spectrometry
Islets of Langerhans are the endocrine portion of the pancreas responsible for maintaining glucose homeostasis through the regulated secretion of numerous hormones, most notably insulin. With highly sensitive analytical methods pioneered almost 25 years ago, the automated measurement of insulin release with a time resolution of seconds from a single islet can be performed. These single endocrine units display complex hormone secretion patterns consisting of rapidly changing phases and oscillations. Despite the presence of hundreds of thousands of these islets in vivo, these complex patterns are also manifested in the blood stream and are critical for proper glucose utilization.

In this talk, various analytical approaches that enable monitoring of insulin secretion from single islets with a time resolution appropriate to discern these various patterns will be discussed. The ability to make intracellular Ca\(^{2+}\) measurements simultaneously with insulin measurements for investigating stimulus-secretion coupling will also be shown. Finally, a mechanism of how populations of individual islets can synchronize their hormonal pulses to produce coordinated oscillations, similar to those found in vivo, will be proposed.

Abstract Text

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

Application Code: Bioanalytical

Methodology Code: Microfluidics/Lab-on-a-Chip
Signaling peptides regulate neuronal circuit activities and a wide range of physiological processes. Measuring the dynamic changes of these important chemical messengers under different physiological conditions enables uncovering their potential functions. Equipped with high-resolution accurate mass (HRAM) Orbitrap instrumentation coupled with various separation techniques and isotopic and isobaric labeling strategies, we aim to explore peptidomic changes in the regulation of feeding behavior. To directly monitor feeding-induced changes in neuropeptide expression levels within the nucleus accumbens (Acb), we employed a combination of cryostat dissection, heat stabilization, neuropeptide extraction and quantitative neuropeptidomics, in rats either anticipating food or having recently completed a meal. Over 300 feeding-related neuropeptides were identified in rat Acb. Feeding altered expression levels of multiple neuropeptides of different families, especially opioid peptides such as enkaphalins and dynorphin, and non-opioid peptides including ProSAAS, orexin, and neuropeptide Y. We further investigated the regulatory functions of novel non-opioid neuropeptides from the ProSAAS family by infusing identified ProSAAS neuropeptides directly into the rat Acb, and monitoring spontaneous feeding and exploratory-like activity. Furthermore, we combined the HRAM multiplexed mass spectrometric imaging (MSI) method with in vivo microdialysis sampling technique to allow monitoring of trace-level neuropeptide secretion, small signaling molecule dynamic changes in the crustacean hemolymph. Finally, the dynamic degradation profiles of neuropeptides were investigated via in vivo microdialysis coupled to HRAM multiplexed MSI on a MALDI LTQ Orbitrap platform. This novel approach enabled direct interrogation of multiple neuropeptide degradation products over an extended period of time and provided novel insight into peptide function and peptide multiplicity.

Keywords: Bioanalytical, Mass Spectrometry, Neurochemistry, Quantitative
Application Code: Neurochemistry
Methodology Code: Mass Spectrometry
Background: Patients with Cystic Fibrosis (CF) are prone to polymicrobial colonization of the airways due to the presence of thick and immovable mucus. Clinical culturing focuses on targeted opportunistic pathogens, takes days to obtain results, and is not able to detect periods of worsened symptoms, leaving physicians to make choices about antibiotic treatment based on trial and error. We are using a multi-omics approach to characterize the microbial community composition and activity of sputum and breath samples in CF patients. An important goal is to identify biomarkers of infection, and link metabolites with the genes and microbes that produced them.

Materials: Induced sputum and breath samples were collected to simultaneously probe: (i) breath gas and sputum metabolites using GC-MS and LC-MS, (ii) microbial taxonomic and functional profiles using metagenomic and 16S rDNA amplicon sequencing, and (iii) active microbial metabolism with model cultures.

Results: Metabolites including 2,3-butanedione and acetaldehyde in breath and 2,3-butanediol, putrescine, and 5-aminovaleric acid in sputum correlate with periods of worsened symptoms, and with the abundance of specific taxa. In addition, a large fraction of molecules detected from sputum with GC-MS correlate well with microbial community composition data, while a large fraction of molecules detected by LC-MS correlate well with patient data such as age.

Conclusion: Metabolites that are uniquely produced by particular microbes are good markers for the active microbes in a disease state. Some metabolites (e.g. fermentation products) are only produced under certain conditions, and thus provide insight into the local environment (e.g. pH, oxygen and nutrients). We have preliminary evidence that suggests microbial fermentation products such as 2,3-butanedione may be important indicators of periods of worsened symptoms. They may also increase microbial virulence and trigger the host immune response.

Keywords: Bioinformatics, Data Analysis, GC-MS
Application Code: Biomedical
Methodology Code: Gas Chromatography/Mass Spectrometry
Technical advances are making it possible to create tissue microenvironments on platforms that are compatible with high-content screening strategies. We have developed microfabricated devices to enable culture of organized cellular structures which possess much of the complexity and function of intact intestinal tissue. Stem-cell culture now enables single stem cells or intestinal crypts isolated from primary mouse or human intestine to grow and persist indefinitely as organotypic structures containing all of the expected lineages of the intestinal epithelium. Our microengineered arrays and fluidic devices allow prolonged culture and experimental manipulation of these organotypic cultures. Millimeter-scale primary intestinal epithelium closely mimics the polarized 3D in vivo microarchitecture of primary tissue. These arrays can be interrogated by any of a variety of techniques including fluorescence, immunohistochemistry and genetic analyses. These bioanalytical platforms are envisioned as next generation systems for high-throughput assays of microbiome-, drug- and toxin-interactions with the intestinal epithelia.

Keywords: Bioanalytical, Biomedical, Biosensors, Biotechnology
Application Code: Biomedical
Methodology Code: Microfluidics/Lab-on-a-Chip
### Abstract Text

The ecology of the gastrointestinal gut shapes human-microbe interactions. Integrated omics which involves the joint analysis of metagenomic, metatranscriptomic, metaproteomic and metabolomic data offers the prospect of comprehensive insights into community composition, physiology, ecology and evolution in situ. Particularly, time-resolved analyses allow the resolution of system-level properties. We have developed an integrative workflow comprising wet- and dry-lab methodologies to enable systematic measurements of microbial communities over space and time as well as the integration and analysis of the resulting multi-omic data. By resolving multi-omics data at the population-level, we have found that in type 1 diabetes different microbial functions, e.g. thiamine biosynthesis, are differentially expressed in the absence of clear taxonomic differences and that the expression of such genes is affected by the abundances of enzymes secreted by the exocrine pancreas. Importantly, specific microbial functions can be encoded and expressed by distinct microbial populations in different individuals. By integrating information from genome to metabolome, integrated omics allows the deconvolution of structure-function relationships by identifying key members and functionalities. Subsequent hypotheses are tested in a recent developed human-microbial co-culture system called HuMiX, which allows the probing of the functional responses elicited by human cells when communicating with gut microbes. Overall, the generated knowledge with respect to host-microbe interactions will further our understanding of the role of the microbiome in affecting human physiology in the context of health and disease.

### Keywords
- Environmental/Water
- Extraction
- Gas Chromatography/Mass Spectrometry
- Metabolomics

### Application Code
- Biomedical

### Methodology Code
- Data Analysis and Manipulation
Microbial natural products represent a critical set of signaling molecules that mediate inter-microbial communication as well as interactions with plant or animal hosts. Natural product discovery efforts have focused primarily on microbial biosynthetic gene clusters (BGCs) containing large multi-modular PKSs and NRPSs; however, sequencing of fungal genomes has revealed a vast number of BGCs containing smaller NRPS-like genes of unknown biosynthetic function. Using NMR spectroscopy- and mass spectrometry-based comparative metabolomics, we show that a BGC in the human pathogen Aspergillus fumigatus named fsq, which contains an NRPS-like gene lacking a condensation domain, produces several novel isoquinoline alkaloids, the fumisoquins. These compounds derive from an unprecedented carbon-carbon bond formation step, which is followed by a sequence that is directly analogous to the biosynthesis of plant isoquinoline alkaloids. Fumisoquin biosynthesis requires the N-methyl transferase FsqC and the FAD-dependent oxidase FsqB, which represent functional orthologs of coclaurine N-methyl transferase and berberine bridge enzyme in plants. Our results show that BGCs containing incomplete NRPS modules may reveal new biosynthetic paradigms and suggest that plant-like isoquinoline biosynthesis is widespread in fungi.
The human lung is a complex multikingdom environment, comprised of fungal spores, bacteria, viruses, and multiple types of human cells (such as airway epithelial cells, fibroblasts, and immune cells). To better understand the interactions among the microbial and human players, we have developed new microscale tools, using the principles of open and suspended microfluidics. Our devices provide for controlled, segmented multiculture environments (2-20 μL in volume) to better understand the chemical signals exchanged between bacteria and fungi in microenvironments that capture salient features of the human lung, including varied extracellular matrix and soluble factor environments. Importantly, we have developed methods to integrate our microscale culture platforms with metabolomics workflows, such as incorporating microscale liquid-liquid extraction as a sample preparation step prior to mass spectrometry analysis. Further, our open microfluidic tools combine functional, phenotypic readouts in parallel with metabolomic analysis to establish links between chemical signals exchanged and their downstream biological functions.

**Keywords:** Bioanalytical, Environmental/Biological Samples, Lab-on-a-Chip/Microfluidics, Liquid Chromatograph

**Application Code:** Bioanalytical

**Methodology Code:** Microfluidics/Lab-on-a-Chip
Trace explosives detection in public spaces requires the use of eye-safe laser wavelengths. We have built a broadband laser system based on multi-soliton generation in photonic crystal fiber driven by a 2 MHz repetition-rate industrial fiber laser with central wavelength at 1550nm, and configured it to detect coherent anti-Stokes Raman scattering for high-speed for high-speed imaging. Experimental measurements were carried out on a number of chemicals including explosives on a variety of surfaces at low-microgram per cm² concentration. Chemically specific images were collected at 0.06 ms per pixel rate. Results from this effort indicate the combination of modern industrial fiber lasers and nonlinear optical spectroscopy should be considered for next generation eye-safe trace explosive detection.
Raman bands in the low energy region of the spectrum of crystals are attributed to so-called external lattice vibrational modes. The Raman bands from these low energy phonons are very sensitive to crystal structure and to chemical bond interactions within the crystal. The low energy vibrational modes of many organic molecular crystals have very high Raman scattering cross-sections. Raman spectra and images of low energy phonons in so-called two dimensional (2D) crystals such as few-layer MoS2 reveal spatial variations in the solid state structure that are not evident in the higher energy bands. We will discuss the sensitivity of low-energy phonons to the structure of 2D crystals, complementarity of reflected light and low-energy Raman imaging, and the probing of 2D layer orientation and stacking through the Raman band structure of low-energy phonons.

Keywords: Material Science, Microspectroscopy, Nanotechnology, Raman Spectroscopy
Application Code: Material Science
Methodology Code: Vibrational Spectroscopy
Raman spectroscopy is ideally suited for planetary exploration, because Raman spectra provide detailed molecular and structural information, very useful for geochemical measurements and for measuring organic and inorganic biomarkers in the search for past or present life on other planets. Traditional grating based dispersive UV spectrometers are typically large and have very low light throughput, not ideal for planetary or on-line applications.

We are developing a new type of Fourier transform (FT) Raman spectrometer; the spatial heterodyne Raman spectrometer (SHRS), which provides high spectral resolution in a very small system with high light throughput. Initially designed with planetary exploration the SHRS is also ideal for on-line and in-situ monitoring. The basis of the SHRS is a spatial heterodyne spectrometer, a type of dispersive interferometer with no moving parts. The SHRS has very high light throughput and provides very high spectral resolution in a very small package. The SHRS also has a very wide field of view simplifying alignment in standoff Raman and allowing the use of extended illumination sources.

In this paper a description of the UV-SHRS system will be given with new results showing deep-UV standoff Raman measurements and an improved UV SHRS that uses a plate beam splitter and compensator plate. Progress toward miniaturizing the SHRS will be shown including the use of diffraction gratings as small as 2.5 mm and the use of a very small low-cost CCD detector as well as a cell-phone camera as detector. Future research will be described related to the design of a standoff SHRS for a SmallSat-sized, 10x10x10 cm, planetary Lander.

**Keywords:** Instrumentation, Raman Spectroscopy, Vibrational Spectroscopy

**Application Code:** Other

**Methodology Code:** Vibrational Spectroscopy
Advances in Raman Spectroscopy

Recent Advances in SERS and TERS

First, I will provide some background material on the basic physical concepts underlying surface-enhanced Raman spectroscopy (SERS) and tip-enhanced Raman spectroscopy (TERS). Next, I will describe recent experiments on: (1) ultraviolet SERS; (2) the application of SERS to the study of atomic layer deposition (ALD); and (3) new insights into the distance dependence of SERS. In the area of TERS, I will talk about new results probing the spatial resolution of ultrahigh vacuum TERS and the application of TERS to the study of electrochemistry at the single molecule level.

Keywords: Raman Spectroscopy, Surface Enhanced Raman Spectroscopy, Vibrational Spectroscopy

Application Code: Nanotechnology

Methodology Code: Vibrational Spectroscopy
As the complexity of biopharmaceutical drugs continues to evolve, so is the need for sensitive tools to help with characterization of these drugs. The testing is needed for innovator drugs but even more importantly for generic versions known as ‘biosimilars and biobetters’. But as these drugs enter the mainstream, another problem emerges – counterfeits.

Among many techniques available for structural studies, Raman spectroscopy, although a well-established technique in analytical sciences, is now finally gaining some popularity. Its advantage is ability to detect the conformation of disulfide bonds and gain information from side-chains, in addition to secondary structure. More recently, several studies have shown an enhanced sensitivity of ROA (Raman Optical Activity) with differences observed when none are observed with any other spectroscopic techniques. And for counterfeits, new studies emerge demonstrating how a handheld instrument with excitation at 532 nm is able to distinguish variety of different counterfeits of best selling biologics.

In this presentation, we will discuss advances in Raman and ROA as applied to structural studies of proteins.

Keywords: Biopharmaceutical, Biospectroscopy, Characterization, Portable Instruments
Application Code: Pharmaceutical
Methodology Code: Vibrational Spectroscopy
Along this century we have witnessed a change in research paradigms from hypothesis based studies to comprehensive analysis of differential changes in a given situation. Those changes lead to generate new hypothesis frequently never foreseen, that is the aim of the so called “omics” technologies.

One of the limitations of multi-omics analysis is the usually limited amount of the sample available for the study. To overcome this drawback our group has been working in different approaches with in vial extraction of minute amounts of sample to expand the amount of information that can be obtained.

In a first study [1] an in-vial dual extraction (IVDE) method was developed that showed the total number of features recovered to be over 4500 from a single 20 μL plasma aliquot. After a one-step extraction consisting of a lipophilic and hydrophilic layer within a single vial insert, the two phases in the vial underwent LC-MS analysis on individually customized LC gradients. Afterwards, Legido’s group [2] optimised an IVDE method, with reversed phase and HILIC chromatography which reproducibly measured over 4,000 metabolite features from as little as 3 mg of brain tissue and explored the opportunities to use dried serum, urine, and cerebrospinal fluid spots [3]. Recently a novel use of IVDE was proposed [4] where three fractions are formed: proteins pellet in the bottom, aqueous-methanol phase with polar compounds and upper ether phase with lipophilic compounds. Entire sample preparation is performed within an insert in a HPLC vial and the sample is injected by adjustment of the height of the injection needle. Lipids and proteins are analysed in the same sample.

References

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

Application Code: Bioanalytical
Methodology Code: Liquid Chromatography/Mass Spectrometry
Organic solvent precipitation is currently the most common sample preparation method for global metabolomics of human plasma using LC-MS because of its wide metabolome coverage and excellent repeatability. However, the two main disadvantages of solvent precipitation are that low abundance metabolites are routinely not detected and high potential for matrix effects. The major objective of this work was to investigate and design new sequential extraction protocols that would increase metabolite coverage of human plasma without increasing LC-MS analysis time versus standard protocols. We investigated four different types of approaches: liquid-liquid extraction with solvents or ionic liquids, solid-phase extraction, and dispersive solid-phase microextraction using functionalized hydrogel microparticles in combination with reversed-phase and mixed-mode LC-MS separation. The MS analyses were performed on Agilent iFunnel 6550 quadrupole-time-of-flight instrument or LTQ-Orbitrap Velos from Thermo Scientific. The data processing was performed using Mass Profiler Professional or Sieve software, respectively. Only metabolites present in minimum 5 out of 6 extraction replicates were included in the comparison. The methods were compared in terms of metabolite coverage, method precision and recovery.

The results show clearly that the optimized sequential extraction protocol that combines liquid-liquid extraction and solid-phase extraction provided 2-fold improvement in metabolite coverage. Ionic liquids also performed very well for lipid removal from the samples, which resulted in almost 2-fold improvement in coverage of intermediate polarity metabolome. Overall, these results demonstrate the important role that sample preparation and new extraction materials play in the state-of-the-art metabolomics.

Keywords: Liquid Chromatography/Mass Spectroscopy, Sample Preparation, Metabolomics, Metabonomics
Application Code: Genomics, Proteomics and Other 'Omics
Methodology Code: Sampling and Sample Preparation
CF is the most common fatal autosomal recessive disorder in North America. A two-tiered strategy is widely used for population-based CF screening using an algorithm based on elevated IRT followed by a DNA mutation panel. Limitations of this screening strategy include a high rate of false positives and the identification of carriers with CFTR allele variants who do not express the disease. Herein, untargeted metabolite profiling of retrospective dried blood spots (DBS) was performed to discover novel metabolic signatures in affected CF newborns as a way to enhance overall screening performance that is also amenable to low cost MS/MS analysis. Retrospective analysis of dried blood spot extracts from 54 normal birth weight newborn infants at Newborn Screening Ontario, were selected as a training set for biomarker discovery. Multisegment injection-capillary electrophoresis-mass spectrometry (MSI-CE-MS) was used for untargeted yet high-throughput screening of polar/ionic metabolites with quality assurance. Over 75 cationic metabolites were consistently detected in DBS extracts with acceptable reproducibility using MSI-CE-MS under positive ion mode detection without system bias based on repeated analysis of pooled quality controls. There was good discrimination in affected CF infants relative to healthy controls based on a sub-set of eight statistically significant metabolites measured in DBS extracts after Bonferroni correction (p < 7.0 E-4). Two novel metabolite adducts elevated in CF infants were tentatively identified and subsequently confirmed in DBS extracts from adult CF cases. A validation cohort is currently underway to evaluate the screening performance of lead metabolites as specific markers for pre-symptomatic diagnosis of CF in affected infants relative to unaffected cases. This work will improve the overall positive predictive value and cost effectiveness of CF screening while reducing widespread genetic testing and undue parental stress.

Keywords: Bioanalytical, Capillary Electrophoresis, Clinical Chemistry, Mass Spectrometry
Application Code: Biomedical
Methodology Code: Capillary Electrophoresis
Metabolomics research requires accurate relative quantification of a large number of metabolites in comparative samples. One approach of performing metabolomic profiling is to use differential chemical isotope labeling (CIL) of metabolomic samples, in combination with high resolution LC-MS, for relative metabolite quantification. In this approach, a control sample is prepared by mixing small aliquots of individual samples to form a pool, followed by heavy-isotope-reagent labeling. This heavy-labeled control is spiked into all the light-isotope-labeled individual samples and thus serves as a global internal standard. The light-labeled metabolite and its corresponding heavy-labeled counterpart in a light/heavy-mixture are detected as a peak pair in MS and their peak area ratio reflects the concentration difference of the metabolite in an individual sample vs. the control. Since the same heavy-labeled control is used for preparing all individual mixtures, the peak ratio values can be used for relative quantification of individual metabolites in different samples. In this presentation, recent advances in labeling chemistries targeting different submetabolomes, separation methods of labeled metabolites, data processing protocols, and metabolite identification strategies will be discussed. A few selected applications of this technique in cellular metabolomics and biomarker discovery will be described.

Keywords: Bioanalytical, Derivatization, Liquid Chromatography/Mass Spectroscopy, Metabolomics, Metabono
Application Code: Bioanalytical
Methodology Code: Liquid Chromatography/Mass Spectrometry
Background
In untargeted metabolomics by LC-MS or GC-TOF MS, often less than 30% of the compounds are identified. Without structural identifications of these molecules we cannot reveal biological mechanisms, biochemical pathways or origins of exposome compounds. While we have to build larger public mass spectral libraries, we will never have authentic mass spectra of all small molecules from reference compounds. The only way around this problem is to predict, score and validate spectra by in silico fragmentation software.

Methods
Our center has developed software for untargeted metabolomics, starting from over 200,000 predicted lipid spectra in LipidBlast over releasing MS-DIAL for untargeted data processing and finally MS-FINDER for computationally identifying novel compounds. The past three years has seen large improvements in MS/MS predictions. CFM-ID, MS-FINDER, Magma+ and the updated MetFrag software have been used in the international CASMI competition for identifying unknowns in LC-MS research. While our center has participated with two groups in this competition, we have now benchmarked and compared the outcomes.

Results
We used all 520 MS/MS spectra given by the CASMI organizers to compare pure in-silico methods by themselves (‘challenge category 2’) versus a combination and ranking of the four methods in addition to using MS/MS spectral match and database queries (‘challenge category 3’). Our results show that using metadata, including mass spectral matching scores, still have an overarching impact on the overall accuracy of ranks. Impressively, such combinations yielded more than 85% correct hits as first candidate for these 520 challenge spectra, giving confidence that identification of unknowns by modern cheminformatics tools is an achievable aim – as long as compounds detected in metabolomics are actually ‘known’ compounds that are part of existing databases such as the 60 million chemicals in PubChem.
Environmental/occupational exposure scientists need a tool for collecting near-real-time personal multi-VOC measurements of industrial workers’ exposures. No such instrumentation currently exists. In this presentation we will describe the first wearable gas chromatographic microanalytical system (µGC) for near-real-time recognition and quantification of volatile organic compounds (VOC) in moderately complex mixtures encountered in working environments. We refer to this instrument as a Personal Exposure Monitoring Microsystem (PEMM). The battery powered µGC prototype measures 20×15×9 cm, weighs just 2.1 kg, has 3-D printed packaging, an on-board helium canister, and it can be worn on the belt of a worker to measure VOCs every 5-10 minutes for several hours. Novel features of the discrete DRIE-Si/Pyrex micro-component chips include a dual-adsorbent µpreconcentrator/focuser (µPCF) with split-flow injections as narrow as 600 ms for benzene and 900 ms for n-dodecane, a 6-m long segmented separation µcolumn with zone-heating for power efficient separations that yielded a 29% reduction in energy per analysis by judicious heater scheduling guided by band trajectory modeling; and a fluidically redesigned array of µchemiresistors (µCR) with gold-thiolate-monolayer protected nanoparticle (MPN) interface films that provide ~ng limits of detection (LOD) from 5-10 mL air samples and sufficiently diverse response patterns for recognizing eluting VOCs. In our first tests of the complete prototype, we demonstrated reproducible analyses of mixtures of up to 9 VOCs in 2.5 min (sampling and analysis). We have just embarked on mock field testing, with good results for the first ternary VOC mixture we monitored repeatedly at ppm levels over time.

Keywords: Environmental/Air, Gas Chromatography, Lab-on-a-Chip/Microfluidics, Volatile Organic Compounds
Application Code: Industrial Hygiene
Methodology Code: Integrated Sensor Systems
Integrated Microscale Chemical Analyzers

**Hand-Portable Liquid Chromatography for Target Chemical Analysis**

Hand-portable liquid chromatography (LC) instrumentation has been slow to be developed and deployed, primarily because it has not been feasible to utilize miniature mass spectrometry (MS) detection for positive identification of target analytes. Unfortunately, ultraviolet absorption (UV) spectra are not specific enough for unequivocal target compound detection/identification, especially for trace detection. However, UV absorbance measurements at two or more wavelengths, combined with chromatographic retention data can be used to provide reliable identification when compared against data acquired from actual target reference compounds. A compact nano-flow LC system was developed that provides chromatographic retention data from two simultaneous separations having different stationary phases and/or mobile phases, both with dual-wavelength (260 and 280 nm) UV-absorption detection. Light-emitting diodes provide sensitive on-column detection for packed and monolithic fused silica capillary columns (150 micron internal diameter). Ratios of retention and absorption data obtained from these simultaneous analyses can be combined to give sufficient information for positive identification of target analytes.

**Keywords:** Capillary LC, Environmental Analysis, HPLC Detection, Portable Instruments

**Application Code:** Environmental

**Methodology Code:** Liquid Chromatography
Recent advances in mid-infrared (3-15 µm) thin-film waveguide technology and on-chip photonics facilitate next-generation label-free chem/bio sensor and assay platforms. In combination with efficient light sources such as tunable quantum cascade and interband cascade lasers (QCLs, ICLs), compact yet robust MIR diagnostics may be conceived. By expanding the range of available MIR transparent waveguides using alternative materials such as suitable semiconductors, diamond, etc. readily serving as thin-film waveguides, ring resonators, disc resonators, slot waveguides or solid-state interferometers the ‘optical gap’ between advanced MIR light sources and detectors may be closed facilitating hybrid or even monolithic on-chip integration of mid-infrared sensing devices towards versatile MIR lab-on-chip systems.

Keywords: Bioanalytical, Biomedical, Infrared and Raman, Lab-on-a-Chip/Microfluidics
Application Code: Bioanalytical
Methodology Code: Vibrational Spectroscopy
Microfabricated capillary electrophoresis devices where first demonstrated over two decades ago. We have recently demonstrated such devices with unprecedented separations efficiency while also monolithically integrating a nano-electrospray emitter. These devices have been used to analyze small molecules, such as amino acids and metabolites, peptides, and proteins as large as 150 kDa. We have also monolithically integrated additional functionality such as solid phase extraction on these devices. In the case of hydrophobic peptides, concentration detection limits have been lowered almost three orders of magnitude. Moreover, our group has been developing miniaturized ion trap mass spectrometry systems that include microfabricated parts with critical dimensions on the micrometer length scale. Ion trap mass analyzers have attractive scaling laws as dimensions are reduced; mass resolution is fundamentally invariant with length scale and charge capacity scales with linear dimension. These ion traps have fundamental dimensions in the 100 to 500-µm range. These mass analyzers have also been demonstrated to operate at unprecedented pressures; beyond 10 Torr. As a natural progression, we have mated these two microfabricated technologies to demonstrate very compact systems that perform high-performance liquid-phase separations with mass spectrometry detection and identification. Example applications will include the analysis of bioreactor broth for amino acids and the analysis of whole blood for amounts of glycated hemoglobin.

**Keywords:** Capillary Electrophoresis, Lab-on-a-Chip/Microfluidics, Mass Spectrometry

**Application Code:** Bioanalytical

**Methodology Code:** Mass Spectrometry
In the last decade an incredible number of highly selective 'first order' analytical technologies have undergone commercial miniaturization to handheld form-factors: Raman, FT-IR, near-IR, XRF, LIBS, and mass spectrometry. The new analytical opportunities for these systems continue to expand rapidly, but most of the opportunities are to provide analytically advanced informing power to non-experts. In these cases, miniature instrumentation is only half the battle. Successful handheld analyzers need to achieve very advanced levels of automation to accommodate situational and operational variables that simply don't exist in a laboratory environment. Furthermore, since there is no technical expert directly involved in the process to make judgements from instrumental data (and in fact subjectivity is problematic in many circumstances), a statistical framework is required that has really not be explored in the academic chemometrics or analytical literature. This talk will review the challenges presented from an analytics/decision theory perspective using examples from fielded handheld Raman, FTIR and mass spectrometry, and the formal chemometric & statistical approaches that have been developed to address these needs.

**Keywords:** Chemometrics, Data Mining, Mass Spectrometry, Portable Instruments

**Application Code:** Safety

**Methodology Code:** Mass Spectrometry
Neurotransmitter signaling provides the chemical messages that allow cells to relay information within a circuit. Glutamate is the primary excitatory neurotransmitter in the brain, but its measurement has been challenging because it is non-electroactive and tightly-regulated in the synapse. We have developed a microelectrode array, electroenzymatic glutamate biosensor that affords near-real time (<1s temporal resolution), sensitive, selective, and spatially precise measurement of glutamate concentration changes in the brains of freely-behaving rodents. We have applied this technology to understand the function of excitatory glutamate signaling within the amygdala-cortical circuits that regulate decision making. Our recording results suggest that glutamate transmission in this circuit occurs when subjects update their reward expectations and then later use this information to guide their reward-seeking decisions. The biosensor technique was combined with chemogenetic pathway-specific interference methods to provide a complementary causal analysis to the correlational recording approach.
Opioid peptides are critically involved in a variety of physiological functions necessary for adaptation and survival, and as such they show tremendous promise as therapeutic targets. However there is a critical gap in understanding when and where these molecules are released, because there is a paucity of detection methods for monitoring opioid peptides in the extracellular space. We have designed a novel waveform that employs two distinct scan rates in each voltammetric sweep to detect enkephalin dynamics in live tissue with sub-second temporal resolution using carbon fiber microelectrodes. Combining two scan rates in a single voltammetric scan is unprecedented in molecular monitoring. It exploits fundamental principles to offer a combination of temporal and spatial resolution, sensitivity, and chemical selectivity that has the potential to advance the voltammetric detection of many classes of molecules. Electrochemical detection is further enhanced by combining this approach with a microelectrode sensing substrate engineered entirely of multi-walled carbon nanotubes spun into a yarn, replacing the standard carbon fiber altogether. The high aspect ratio and distinct electronic properties of the nanotubes result in markedly improved selectivity, sensitivity, and spatial resolution, as well as faster electron transfer kinetics that are manifested as sharper voltammetric peaks when compared to conventional carbon-fiber microelectrodes. By combining a groundbreaking voltammetric approach with a novel tool engineered from nanoscale materials, we are enabling a shift from interferential measures of endogenous opioid activity to direct molecular measurements.

Keywords:  Bioanalytical, Microelectrode, Neurochemistry, Voltammetry
Application Code:  Neurochemistry
Methodology Code:  Electrochemistry
Serotonin neurotransmission is implicated in a multitude of the brain’s functions, however the exact functions played by this messenger remain unknown. We and others have unearthed several enigmatic pieces of information about this neurotransmitter. For example, extracellular serotonin levels are profoundly controlled by many, synergistic regulatory mechanisms because high extracellular serotonin levels are potentially fatal. Paradoxically, mice genetically depleted of serotonin do not display significant pathophysiology. Additionally, serotonin neurons display a unique propensity to regenerate after damage. In this work, we show how fast scan cyclic voltammetry and fast scan adsorption controlled voltammetry can be utilized to provide critical physiological information about this important neurotransmitter. We probe serotonin neurotransmission in 3 different brain regions. Using pharmacology and mathematical analyses we show that voltammetric signals provide critical information about the differences in extracellular mechanisms that regulate serotonin between different brain regions. Our study highlights the importance of voltammetry for deciphering the fundamental mechanisms that regulate serotonin [i]in vivo[/i].
Carbon-fiber microelectrodes can be used to reduce molecular oxygen, and, when probed with cyclic voltammetry, a unique identifier for oxygen is obtained. This approach has been used to explore oxygen supply during glutamatergic neurotransmission as well as during spreading depression.

Keywords: Microelectrode, Voltammetry
Application Code: Neurochemistry
Methodology Code: Electrochemistry
Local neurochemical disturbances occurring after brain injury provide important information about the ability of the nervous tissue to recover from the initial insult. Monitoring the chemical composition of the brain interstitial fluid is therefore an important challenge for both pre-clinical and clinical research on brain injury. Microelectrode biosensors are a promising technique to monitor the brain with a temporal resolution in the order of seconds. Here, ultra-microelectrodes based on platinized carbon fibers were fabricated to obtain biosensors with less than 15 µm external diameter. Platinization was achieved by sputtering a 10 nm Cr adhesion layer followed by 100 nm of platinum. Platinized carbon fibers were then encased in a glass micropipette and covered with electropolymerized poly-phenylenediamine for selectivity, and covalently immobilized oxidase enzymes (glucose oxidase, lactate oxidase, D-amino acid oxidase or glutamate oxidase). After implantation in the rat parietal cortex, such biosensors detected (1) a lower basal lactate concentration, (2) a slower diffusion of glucose and D-serine through the blood brain barrier, and (3) a smaller lactate response to cortical spreading depolarization. The concentrations estimated by the small biosensors based on platinized carbon fibers, and their dynamic changes across time, were significantly different from the estimates provided by more conventional biosensors with 100 µm external diameter. We conclude that such biosensors avoid major mechanical injury to blood vessels, preserve the blood brain barrier at the site of implantation, and therefore, provide more accurate measurements from the brain interstitial fluid.

**Keywords:** Bioanalytical, Biosensors, Microelectrode, Neurochemistry

**Application Code:** Neurochemistry

**Methodology Code:** Electrochemistry
Trapping light into gaps between noble metal nanostructures yields intense local amplification of the optical field, which opens up new regimes of chemical analysis. The largest enhancements come from the smallest nano-gaps, and recently we have devised a whole class of approaches yielding robust, low-cost, reproducible, sub-nm gaps that produce exceptional performance for surface-enhanced-Raman scattering, capable of looking at down to individual molecules over periods of many minutes. We show that we are also able to optically control the position of individual atoms, producing optical cavities with volumes much less than a nm on each side, a billion times smaller than the wavelength scale for visible light. This enables us to watch individual bonds between molecules in real time. We are also able to explore larger-scale robust nanoassembly to track neurotransmitters in real biofluids such as urine without biofouling, and are exploring the bioscreening that this is capable of delivering.

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Keywords: Bioanalytical, Nanotechnology, Surface Enhanced Raman Spectroscopy, Vibrational Spectroscopy
Application Code: High-Throughput Chemical Analysis
Methodology Code: Molecular Spectroscopy
Super-resolution imaging is a far-field optical microscopy technique, which allows the diffraction limit of light to be overcome through the combination of photoswitchable optical probes and single molecule localization techniques. This talk describes the use of super-resolution imaging for studying the position of single ligands bound to the surface of single plasmonic nanoparticles as well as providing insight into how plasmon coupling effects impact the accuracy of single molecule localization.

Keywords: Molecular Spectroscopy, Nanotechnology
Application Code: Nanotechnology
Methodology Code: Sensors
Metallic nanoparticles have found their way into a wide range of applications, from photocatalysis to biomedicine and chemical sensing. In particular, the light-matter interaction called localized surface plasmon resonance (LSPR) has attracted much attention because of its ability to enhance spectroscopic signals and detects changes in local environment. The performance of plasmonic nanostructures strongly depends on their ability to concentrate the electric field of light in sub-wavelength regions at the resonant frequency. By changing the particle size, shape, and composition it is possible to control light confinement and therefore control how particles interact with their environment.

Here, electron-based techniques (electron-energy loss, cathodoluminescence, and energy dispersive X-ray spectroscopy) are used to characterize the field localization and composition in a variety of bimetallic plasmonic systems, including Au/Pd octopods, Pt-decorated Au prisms and Pd-decorated Al spheroids. We show that the particles incorporating catalytically active but heavily damped metals nevertheless sustain multiple size-dependent LSPRs that are strongly localized at the particle tips or edges, depending on the mode energy. Tomography and composition mapping unravels the availability of catalytic metal at the surface of the particle, providing new catalysis and surface attachment opportunities while maintaining refractive index sensing and surface enhanced spectroscopy capabilities.
High refractive index sensitivity (RIS) is engineered into stellated Au–Pd nanocrystals (NCs) with Oh symmetry through lowering the dielectric dispersion at the NC resonant wavelength with internal or external atomic % Pd. This compositional and structural control is achieved through seed-mediated co-reduction as a new route to multimetallic nanostructures with high symmetry. To our knowledge, these NCs display the highest ensemble RIS measurement for colloids with LSPR maximum band positions $900 \text{ nm}$, and these results are corroborated with simulations by the finite difference time domain method. Finally, the external composition can provide chemical selectivity and multifunctionality for sensor applications.
Abstract Text

In this presentation, the properties of different plasmonic nanomaterials will be discussed in the context of clinical and biological sensing. In our research, we have studied and integrated novel plasmonic materials based on nanoparticle and hole arrays, surface chemistry relying on peptide monolayers and unique instrumental designs for sensing biomolecules of importance in disease detection, monitoring the course of treatment of patients during ongoing therapies or of general interest in biological systems. The plasmonic properties of nanohole and microhole arrays were studied in different excitation modes (transmission and attenuated total reflection) for plasmonic sensing and in surface-enhanced excitation. The nanohole and microhole were arrayed using photolithography on a 4” wafer and the nanohole arrays were integrated in a 96-well plate reader. This platform was used to screen antibodies for PSA sensing and to monitor methotrexate, an anticancer agent used in chemotherapy. We have also developed a SPR and LSPR sensing platform based on a small and portable instrument. Competition assays were validated for therapeutic drug quantitation, such as methotrexate and antibiotics and for monitoring therapeutic responses of patients undergoing leukemia treatments.

Keywords: Bioanalytical, Biomedical, Biosensors, Clinical Chemistry
Application Code: Clinical/Toxicology
Methodology Code: Sensors
The miniature mass spectrometry (MS) analysis systems will serve as a highly effective tool for in-situ, real time chemical and biological analysis. Point-of-car testing is expected to be a major application field for miniature MS systems. A series of technologies have been explored for fast extracting and ionizing target analytes directly from biological samples for both qualitative and quantitative analysis. The goal is to develop disposable sample cartridges that allow analysis with high sensitivity and high specificity using samples of small amounts but without using traditional lab equipment or traditional procedures. Paper spray and extraction spray were used for analysis of dried sample spots, slug flow microextraction was used for liquid biofluid samples, extraction with sampling probe is used for tissue analysis. Polymer-coated probes have been developed for efficient collection of analytes. Real time reactions have also been developed to improve the sensitivity and specificity of the target biomarkers. The combination of the various cartridge-based technologies with the miniature mass spectrometer have been characterized and will be reported.

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

Keywords: Bioanalytical, Mass Spectrometry, Sample Handling/Automation
Application Code: Bioanalytical
Methodology Code: Microfluidics/Lab-on-a-Chip
### Abstract Text

Ambient ionization techniques such as DART, DESI and ASAP have been developed rapidly for the direct sampling of untreated complicated samples. In these techniques, the sample is held in close proximity to the sampling inlet, and a heated gas or an electrically charged mist is used to desorb material from the sample. The gas-phase species are then pulled by the vacuum draw of the MS for detection. However, these techniques are usually limited to the analysis of high volatile small molecules. A simple method to introduce unprocessed samples into a solvent for rapid characterization by liquid introduction atmospheric mass spectrometry (ESI/APCI) has been lacking. Here, the continuous flow open port probe (OPP) sampling interface is introduced to fill this void.

The OPP sampling interface used 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 (by nebulizer gas) and into the ionization source. Simply touching a sample to the liquid in the OPP sampling port introduces the sample into a flowing solvent stream that transports the material into the commercial ionization probe (e.g. APCI, ESI). In contrast to other direct sampling interfaces, with the OPP the sample is transferred to the liquid stream prior to the ionization, allowing a wide species coverage.

Using this OPP sampling interface, rapid, direct sampling and analysis possibilities are exemplified with both solid (e.g. SPME fibers) and liquid samples (e.g. dispensed droplets). High sensitivity (better than 1 ng/mL), good accuracy (> 90%) and linearity (R²>0.998) was observed for various assays. These results demonstrated that OPP could be used as a simple, versatile and high-throughput system to rapidly introduce multiple types of samples into a solvent flow stream for subsequent ionization (e.g. ESI, APCI, APPI, ICP, etc.) and analysis by MS.

### Keywords
- High Throughput Chemical Analysis
- Mass Spectrometry
- Sample Introduction

### Application Code
- High-Throughput Chemical Analysis

### Methodology Code
- Mass Spectrometry
Sampling and Sample Preparation for Direct Introduction Mass Spectrometry

Functionalized Medical Swabs Suitable for Monitoring Allergic Responses in Atopic Patient Using DESI MS

Standard medical ryon swabs are routinely used to sample human mucosa for clinical diagnostics. However, the lack of suitable collection devices for simultaneous sampling, extraction and direct analysis of desired molecules, limits their application for Point-of-Care Diagnostics. Desorption Electrospray Ionization Mass Spectrometry (DESI MS) is an ambient ionisation technique which allows the analysis of molecules from the surface of arbitrary objects. We present a least effort/least-disruption technique for augmenting the information obtained from clinical swab analysis with DESI-MS mucosal metabolite profiling. Ionisation of mucosal biomass occurs directly from a rotated swab. To further improve the extraction efficiency, functionalised swabs with biocompatible HLB or C18 coating were compared with standard ryon swabs by dipping into spiked nasal fluid samples. Nasal mucosa was collected from atopic patients at different time points after initiating an allergic reaction by Timothy grass. Overall, the direct analysis of nasal mucosa results in 300-2000 spectral features including human metabolites, lipids, bacterial metabolites and inflammatory mediators. Functionalized swabs were found to improve sensitivity for hydrophobic analytes including eicosanoids, due to improved selective extraction efficiency of nonpolar analytes from the sample matrix compared to ryon swabs. These results highlight the potential of direct swab analysis by DESI-MS for a wide range of clinical applications including rapid mucosal diagnostics for microbiology and immune responses.

Abstract Text

Keywords: Extraction, Lipids, Mass Spectrometry, Metabolomics
Application Code: Clinical/Toxicology
Methodology Code: Sampling and Sample Preparation
Solid Phase Microextraction-Mass Spectrometry (SPME-MS): Recent Developments and Applications

This new era of mass spectrometry (MS), where sample preparation devices are directly and efficiently coupled to MS instrumentation, has given rise to a growing branch of innovative research where micro/nano-extraction approaches excel. Solid-phase microextraction (SPME), a worldwide recognized green sample preparation technique, was certainly not the exception. In this study, we present diverse SPME-based devices recently developed in our laboratory for the extraction/enrichment of analytes of interest from 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), SPME-nano-electrospray-ionization (SPME-nano-ESI-MS), and Desorption Electrospray Ionization (DESI). Total analysis time did not exceed 5 minutes and sample volumes ranging between 1-1500 \( \mu \text{L} \) 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 pg/mL to sub-ng/mL range were obtained, while good accuracy, and linearity were attained for all the studied probes (i.e. therapeutic-drugs, pharmaceuticals, immunosuppressants, and pesticides) in PBS, urine, plasma, serum, blood, saliva, grape-juice, orange-juice, milk, and ground-water. In addition, this work succinctly describes the development of these technologies and their evolution in terms of geometry, coating characteristics, and thermal/spray stability, as well as its initial application to the study of tissue and homogenized cells.

Keywords: Bioanalytical, Environmental Analysis, Mass Spectrometry, SPME

Application Code: Bioanalytical

Methodology Code: Mass Spectrometry
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. Examples of the application of these devices to the study of fundamental biology will be described. In addition, I will describe the impact this class of microfluidics is having on biotechnology.

Keywords: Genomics
Application Code: Genomics, Proteomics and Other 'Omics
Methodology Code: Microfluidics/Lab-on-a-Chip
We have developed a single molecule detection method for counting individual protein molecules using microwell arrays. This single molecule array (Simoa) method is a digital sandwich assay that enables ultrasensitive detection of proteins and nucleic acids. We have applied the method to the measurement of proteins in single cells. By measuring hundreds of individual cells, we can determine the heterogeneity of the population and also observe rare cells that exhibit an unusual phenotype. We have applied the method to the analysis of cultured cancer cells to characterize the heterogeneity of the population. Such high resolution measurements of individual cells in a population enable the discovery of new biological phenomena and may one day enable the detection of rare cells in a tissue biopsy.

Keywords: Bioanalytical, Enzyme Assays, Proteomics, Single Molecule
Application Code: Genomics, Proteomics and Other 'Omics
Methodology Code: Fluorescence/Luminescence
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 droplet microfluidic technology for high throughput single cell isolation, manipulation, and analysis at the DNA, RNA and protein level with single-molecule sensitivity.
The ability to correlate single-cell genetic and protein expression information to cellular phenotypes will provide the kind of detailed insight into human physiology and disease pathways that is not possible to infer from bulk cell analysis. This capability is particularly pertinent to understanding cancer biology, given the highly heterogeneous nature of the disease. This presentation describes a set of single-cell technologies we developed for deployment in precision medicine.
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. We combine spatial and spectral encoding with ultra-high density antibody microarrays patterned in sub-nanoliter microchambers for co-detection of 42 immune effector proteins secreted from single cells, representing the highest multiplexing recorded to date for a single-cell secretion assay. Using this platform to profile human macrophages stimulated with pathogenic ligands reveals previously unobserved deep functional heterogeneity and varying levels of pathogenic activation. Surprisingly, the subpopulation architecture is highly conserved throughout the cell activation process, collectively mediating the immune homeostasis. A subset of these cells display multiple effector functions, named polyfunctional population, representing the “super warriors” with the greatest potency and durability in immune defense. This technology was also applied to the measurement of cytokines produced from hematopoietic cells in myeloproliferative diseases (MPD). The polyfunctional subpopulation was found markedly increased in disease. Unexpectedly, we found “normal” hematopoietic cells in the bone marrow of MPD also display abnormal cytokine functions, contributing substantially to pathogenesis and therapeutic response. All these results underscore the complexity of phenotypically similar cell repertoire at the functional level. Our technology permits a full-spectrum dissection of the immune functional states at the single-cell level and represents an enabling tool for next-generation clinical immune monitoring.
Column technology in liquid chromatography is an ever-evolving area that has enabled fast, efficient, and selective separation of complex sample mixtures. Despite shortcomings associated with its hydrolytic instability, silica continues to dominate as the solid support material to which the stationary phases are anchored. Reasons for this dominance include factors such as silica’s mechanical strength, it is economical, can be found in relatively high purity, and it is amenable to surface functionalization using well-known silane chemistry. Generally, alternatives to the traditional silane chemistry used to bond stationary phases on silica supports are pursued to increase hydrolytic stability and/or to alter selectivity.

We report on the modification of silica particles using diazonium chemistry to create a thin polymeric layer on the silica surface baring amine functionalities. The modified particulates were characterized by means of IR spectroscopy, elemental analysis, and gas adsorption measurements. The modified particles were packed into chromatographic columns and tested under liquid chromatographic conditions. The packed column showed to be stable under the chromatographic testing used and exhibited both, HILIC and reversed phase adsorptive characteristics. In this presentation, we will discuss the synthetic approach to obtain the aminated layer on the silica surface and its characterization. Preliminary liquid chromatographic assessment of the modified silica and its stability will also be presented.

Keywords: Chromatography, HPLC Columns, Liquid Chromatography, Modified Silica
Application Code: General Interest
Methodology Code: Liquid Chromatography
Experimental studies of the precision of quantitation in two-dimensional liquid chromatography (2D-LC) have shown that its precision is not as good as that of 1D-LC. A number of causes for this disparity including: the larger number of integrations events involved in 2D-LC than 1D-LC, and the phase angle between a sampling event and the first dimension (1D) peak maximum have been studied. No ‘smoking gun’ that fully explains the poorer performance has been found. We will show that the problem is due to the reproducibility of actuating the valve used as a 1D sampling device and to run-to-run fluctuations in the 1D retention time. Our experimental data convincingly show that good precision is possible in 2D-LC. Simulation and theory yield the necessary guidelines to achieve this performance.

Depending of the experimental conditions a portion of the analyte mass eluting from the 1D separation can be lost from the sampling device during its transfer to the 2D separation. Fluctuations in the time between sampling and the 1D peak time become critical. Thus, improving precision requires either reducing analyte losses OR very precise control of the phase angle. When open loops are used for sampling the first dimension the “easy” way to improve the area precision is to deliberately under fill the loops by at least 20%. This will reduce the analyte loss to a level where the imprecision of timing has an insignificant impact on the overall area precision of the 2D-LC method.
Slip flow enables study and application of particles as small as 0.5 um for UHPLC of proteins. Unprecedented plate numbers are generated for these particles when packed in capillaries. Already these give higher peak capacities in RPLC for top-down proteomics, and higher resolution of RPLC, HIC and other modes of separations for proteins drugs. Reducing instrumental contributions to broadening will maximize impact of these efficient columns.
Abstract Text

Typically, we controlled chemistry and through it selectivity of our monolithic columns by using: (i) direct copolymerization of functional monomers, (ii) preparation of parent monolith with reactive functionalities and its post-polymerization functionalization, (iii) thermally or photoinitiated grafting of functional monomers on the pore surface, and various new clones of these techniques. Recently, a new approach was introduced that enables functionalization of pore surface of monoliths and involves application of metal-organic frameworks (MOFs). These frameworks are compounds consisting of metal ions or clusters coordinated to rigid organic molecules to form one-, two-, or three-dimensional structures that can be porous. We used two implementations: (i) admixing preformed MOF to the polymerization mixture followed by the thermally initiated free radical polymerization and (ii) forming the MOF within the pores applying an in-situ approach. The former technique will be demonstrated with the preparation of monolithic column designed for enantioseparation. The latter led to columns for selective preconcentration of compounds from complex mixtures and efficient catalysis.

Keywords: Bioanalytical, Capillary LC, Chromatography, HPLC Columns

Application Code: Bioanalytical

Methodology Code: Liquid Chromatography
Allenes are cumulenes with three contiguous carbons linked together through double bonds. 1,3-disubstituted allenes are not superimposable on their mirror image; as a consequence they are chiral. Chiral allenes are increasingly important in organic synthesis due to their interesting reactivity. Because of their applications in the field of asymmetric catalysis and in the pharmaceutical industry their optical purity is always a parameter which needs to be determined. In this article, we report the enantiomeric separation of hexa-3,4-diene-3-ylbenzene, an aromatic allene, on a cellulose carbamate (Chiralcel OD-3) stationary phase, using heptane as the mobile phase. Spectroscopic studies using infrared (IR) and vibrational circular dichroism revealed that, in the presence of heptane, the stationary phase undergoes conformational change due to intermolecular H-bonding between the CO and NH of the neighboring polymer chains. Van’t Hoff plots for the retention factor, k, showed that the retention of the two enantiomers is dominated by the enthalpy, while the plot for the selectivity is entropy driven. This suggests that the enantioselectivity is a result of inclusion of the enantiomers in the cavities of the chiral stationary phase. VCD spectra, along with density functional theory calculation (DFT) of the interaction between each

**Keywords:** Chiral Separations, Chromatography, HPLC, Spectroscopy

**Application Code:** Other

**Methodology Code:** Liquid Chromatography
There has been much interest around the usage of chlorine-based disinfectants at farm and processing plants following reports of residues contamination in milk and the potential for the development of resistant strains of bacteria. Chlorate and perchlorate (oxychlorine anions) have emerged as the latest problem residues for the dairy industry owing to the development of new analytical methodologies based on liquid chromatography coupled to tandem mass spectrometry. Despite reports of these developments, not many methodologies have been published in peer-reviewed literature for the analysis of oxychlorine residues. Although they are low molecular weight compounds, oxychlorine anions can be selectively detected in milk and dairy powders by new tandem mass spectrometers to low parts per billion levels. However, the development of stable chromatography is the biggest challenge for chlorate and perchlorate analysis. Most methods report the analysis of perchlorate residues in water using ion chromatography, which requires specialized chromatographic hardware that is generally double the cost of regular LC systems. In this paper, we discuss alternative chromatographic systems for the analysis of oxychlorine anions based on specialized phases along with sample preparation approaches that allow the sensitive analysis of residues in milk and dairy products.
Article 9 of the SPS agreement creates a mandate for signatories to offer technical assistance to their trading partners. However, laboratory capacity building initiatives often involve 'point solutions' to address a specific commodity or issue for a given country, or ad-hoc training programs that lack the ability to address on-going problems in a sustainable manner. Also, despite the fact that the purpose of training is to bring about behavioral change, seldom is any consideration given to assessing and monitoring for successful implementation of lessons learned.

Through 2015 the Global Food Safety Partnership (a PPP led by The World Bank) delivered an innovative and scalable lab capacity building activity for more than 40 Chinese scientists using a Training-of-Trainers model and demonstrated the efficacy of the resulting training through proficiency testing.

The PT results demonstrated such unequivocal success that the model is being explored for roll out in other emerging economies.

**Keywords:** Agricultural, Food Safety

**Application Code:** Other

**Methodology Code:** New Method
Österreichische Agentur für Gesundheit und Ernährungssicherheit GmbH (AGES – Austrian Agency for Health and Food Safety) is the leading Agency responsible for minimising healthcare, food safety and food security and consumer protection risks. It is a limited liability company fully-owned by the Republic of Austria, which carries out services based on the Austrian National Regulations and the relevant European Union (EU) directives and decisions.

In 2002, 18 federal institutes from the areas of food safety, public health, veterinary medicine and agriculture joined together under AGES, an Agency in which the government Competent Authorities in various areas dealing with the food supply chain are bundled into one organization. Prior to that government departments responsible for agriculture, food, human medicine, and veterinary medicine were all responsible for only those tasks falling within their own field.

The work of AGES will be portrayed with examples of food safety incidents in Austria and European Union.

Keywords: Food Safety
Application Code: Food Safety
Methodology Code: Education/Teaching
**Session Title**  
Food Safety and Quality: Emerging Challenges

**Abstract Title**  
**Discrimination of Honey of Different Botanical Origins Using an Untargeted High-Definition Metabolomic Workflow**

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**Abstract Text**

Honey is a natural product which possesses therapeutic, nutritional and industrial value. It can be a high value food commodity especially in the case of certain unifloral honeys used in apitherapy due to their healing and antibacterial properties. Honey can also be the subject of fraud, commonly by adulteration using sugars but also by mislabeling or false declaration of botanical origin. The study demonstrated the successful employment of an untargeted high-definition metabolomic approach for the floral classification of honey. Initial investigations were undertaken with 5 commercial (shop bought) honeys of polyfloral origin to ascertain whether they could be differentiated by ion mobility coupled non targeted metabolomic analysis combined with multivariate statistics. Once positively confirmed that this was a successful approach the experiment was expanded to unifloral honeys of authentic verified origins. The honeys were collected over multiple years from 2009-2014 and from multiple countries including Lithuania, Poland, Denmark, New Zealand and Norway. UPLC-HDMSE analysis of the samples was completed in triplicate in both positive and negative ESI in a randomised fashion and the resulting data were processed in Progenesis QI. Unsupervised PCA showed a clear differentiation between the unifloral honeys with Rape and Buckwheat showing the most close association. Further investigations were made with OPLS-DA analysis to elucidate the analytical components responsible for the differences between the honeys. Unique markers of all of the honeys were identified. Initial investigations were made with OPLS-DA analysis to elucidate the analytical components responsible for the differences between the honeys. Unique markers of all of the honeys were identified. Validation of selected identified markers was undertaken using targeted metabolomics on a UPLC Tandem Quad MS. An evaluation of the markers of botanical origin discovered in the non-targeted metabolomic approach were compared to those found using a similar experimental workflow using rapid evaporative ionisation MS (REIMS).

**Keywords:**  
Food Identification, Liquid Chromatography/Mass Spectroscopy, Metabolomics, Metabonomics

**Application Code:**  
Food Identification

**Methodology Code:**  
Liquid Chromatography/Mass Spectrometry
Brazil’s exports of processed meat to USA have been subject to veterinary drugs residue surveillance by the Food Safety Inspection Service (FSIS) of the United States Department of Agriculture (USDA) including ivermectin at a Maximum Residue Limit (MRL) set at 10 µg/Kg in processed meat products. In the European Union (EU) MRL for ivermectin in raw bovine muscle and processed meat is 20 µg/Kg. At its 19th Session (2010), held in Vermont, the Codex Committee of Residues of Veterinary Drugs in Foods recommended a risk assessment study to set a CODEX MRL for non-lactating bovines. Today, CODEX ALIMENTARIUS recommends MRLs of 100 µg/Kg in liver and 20 µg/Kg in fat as the target tissues to be monitored for non-lactating animals.

In the initial FSIS survey a total of 37 non-compliant results were reported. The meat industry in Brazil started certain measures in order to avoid shipments of non-compliant products to the USA and EU. Microbioticos has been participating in this control program, with an efficient system of delivering a high throughput LC-MS/MS analytical service for ivermectin residues using a validated analytical method at a limit of quantification of 2.5 µg/Kg and a turn STIU nd time of less than 24 hours. It involves a simple liquid extraction and clean up procedure on meat and meat product samples. The consequence of this is that Brazil has ivermectin residue levels in meat products below the EU and USA MRL’s. This factor may have contributed to the North American raw meat exports permission recently issued by the United States. Brazil had a big spread of ivermectin misuse in livestock production system and a coordinated effort by the producers, analytical laboratories and the regulators has resulted in successful implementation of effective measures to control ivermectin residues in Brazilian meat products.

Keywords: Liquid Chromatography/Mass Spectroscopy, Monitoring, Toxicology
Application Code: Food Safety
Methodology Code: Liquid Chromatography/Mass Spectrometry
Bioanalytical Methods to Study Neurological Disorders

Widely Targeted Metabolomics Using Derivatization and LC-MS for Neurochemical Study and Biomarker Discovery

Metabolomics offers great potential for biomarker discovery and fundamental studies of neurobiology. Untargeted methods often suffer from poor reproducibility, while targeted methods are typically too narrow in scope. Widely targeted methods combine aspects of both targeted and untargeted approaches to monitor a large number of targeted metabolites, allowing for hypothesis generation and testing without the challenges of metabolite identification.

We have developed a widely targeted method for 70 neurochemicals using liquid chromatography coupled to a triple quadrupole mass spectrometer. This method uses benzoyl chloride derivatization, which is a simple, rapid reaction at room temperature. Derivatization increases retention of polar metabolites for reverse phase chromatography, and increases sensitivity 10-1,000 fold. Additionally, derivatization makes it easy to generate isotopically labeled internal standards for each targeted metabolite, which is essential for accurate quantification in biological samples.

This method was applied to plasma collected from healthy controls and Parkinson's patients to screen for potential biomarkers. Parkinson's disease does not have a diagnostic blood test, so finding plasma biomarkers could allow for earlier diagnosis and treatment. We found several metabolites which differed between the controls and the Parkinson's patients, though these differences were found to be related to treatment with L-dopa, rather than the disease itself. This demonstrates the importance of well controlled studies for biomarker discovery.

To further increase the scope of our work, we have been exploring additional derivatization techniques with complementary reactivity to benzoyl chloride. We are particularly interested in targeting carboxylic acids, which compose a large percentage of the metabolome. Aniline derivatization has shown promise, and alternatives to aniline which may be more reactive are being investigated as well.

Keywords: Bioanalytical, Derivatization, Liquid Chromatography/Mass Spectroscopy, Metabolomics

Application Code: Genomics, Proteomics and Other 'Omics

Methodology Code: Liquid Chromatography/Mass Spectrometry
Advancements in analytical approaches over the past decades have enabled improved chemical characterization of the brain. These gains in analytical capability are important for reduction of knowledge gaps in brain physiology and pathology as well as addressing the corresponding absence of effective treatments for numerous neurological diseases. The ability to assay individual cells is one area where further analytical advances should improve our understanding of brain function, in part due to the functional, structural, and chemical heterogeneity of the organ. In this work, capillary electrophoresis approaches utilizing large-volume sample stacking (LVSS) are developed for the analysis of a variety of small molecules. These sample stacking approaches provide greater than two orders of magnitude enhancement in sensitivity. Variants of sample preconcentration methods amenable to chiral discrimination, nonaqueous separations as well as a variety of detection modalities (ultraviolet absorption, laser-induced fluorescence and mass spectrometry) have been developed and demonstrated to work robustly for the analysis of individual cells. We have successfully applied LVSS for the analysis of D-amino acids (D-AAs) in individual neurons from [i]Aplysia californica[/i], an established neurobiological animal model, despite the challenges of low endogenous analyte abundance and limits on sample volumes. The ability to more effectively measure the levels of D-glutamate, a poorly characterized D-AA in animals, was a direct result of the improved figures of merit of the developed method compared to standard capillary zone electrophoresis. The enhanced sensitivity of methods utilizing capillary-scale separations with online preconcentration appears well-suited for improved molecular investigations of the healthy and diseased brain.

This work was funded via NSF CHE-1111705.

Keywords: Amino Acids, Capillary Electrophoresis, Chiral, Neurochemistry

Application Code: Bioanalytical

Methodology Code: Capillary Electrophoresis
Macrophages can be differentiated into M1 and M2 phenotypes, which act as inflammatory or anti-inflammatory cells, respectively. M1 cells are known to produce large amounts of NO as part of the immune response. Extended M1 activation can lead to the production of reactive nitrogen species (RNS) such as peroxynitrite, which has been linked to neurodegenerative diseases. Therefore, it is crucial to detect RNS in cells of different phenotypes. Previously, the Culbertson group has developed a microfluidic device capable of single cell analysis (SCA). The goal of this research is to couple SCA with electrochemical detection (EC). EC is able to detect all electrochemically active species and easily coupled with microfluidic devices. In order to prevent the large electric field necessary for cell lysis and electrophoresis from causing large electrical noise, a cellulose acetate decoupler was placed in front of the working electrode. To test this system, Jurkat cells were loaded with ascorbic acid and 6-CFDA and run through the device. Ascorbic acid was chosen because it is easily oxidized, simple to load into cells, and important to the redox balance within the cells. 6-CFDA was added to the cells as an internal standard and has been previously detected on the device. The cell lysate was separated by ME and then the analytes were simultaneously detected with EC and fluorescence detection. In the future, this method will be used to separate and detect multiple RNS and antioxidants in phenotype-specific single cell lysates; ultimately providing a better understanding of the role nitrosative stress plays in neurodegeneration.
Bioanalytical Methods to Study Neurological Disorders

Zebrafish as a Model of Chemotherapy Induced Cognitive Impairment

As the use of chemotherapeutics for the treatment of cancer has advanced, the survival rates for cancer have significantly increased, leading to increased interest in the quality of life of patients after treatment. Chemobrain is a neurological syndrome, reported by patients often long after chemotherapy treatment has concluded, that is characterized by decreased cognitive abilities, memory, and concentration. Previously, our lab has reported the impairment of the release of dopamine and serotonin, neurotransmitters that play important roles in cognitive function. Here, we measure sub-second neurotransmitter release and uptake characteristics in chemotherapy-treated zebrafish. Zebrafish offer several potential advantages over rodents, such as easier genetic manipulation, higher throughput, and lower cost. These advantages open the possibility of not only resolving the neurological mechanisms of chemobrain, but also facilitate the evaluation of potential therapies. Zebrafish were treated with both carboplatin or 5-fluorouracil in either their habitat water or with their food. We varied the dose and duration of treatment and measured evoked dopamine release from ex vivo whole brain preparations using fast scan cyclic voltammetry. The total content of dopamine in the brain preparations was studied using a mass spectrometry method. Finally the kinetics of dopamine uptake was studied using simplex modelling techniques.

Abstract Text

As the use of chemotherapeutics for the treatment of cancer has advanced, the survival rates for cancer have significantly increased, leading to increased interest in the quality of life of patients after treatment. Chemobrain is a neurological syndrome, reported by patients often long after chemotherapy treatment has concluded, that is characterized by decreased cognitive abilities, memory, and concentration. Previously, our lab has reported the impairment of the release of dopamine and serotonin, neurotransmitters that play important roles in cognitive function. Here, we measure sub-second neurotransmitter release and uptake characteristics in chemotherapy-treated zebrafish. Zebrafish offer several potential advantages over rodents, such as easier genetic manipulation, higher throughput, and lower cost. These advantages open the possibility of not only resolving the neurological mechanisms of chemobrain, but also facilitate the evaluation of potential therapies. Zebrafish were treated with both carboplatin or 5-fluorouracil in either their habitat water or with their food. We varied the dose and duration of treatment and measured evoked dopamine release from ex vivo whole brain preparations using fast scan cyclic voltammetry. The total content of dopamine in the brain preparations was studied using a mass spectrometry method. Finally the kinetics of dopamine uptake was studied using simplex modelling techniques.

Keywords: Clinical Chemistry, Electrochemistry, Microelectrode, Neurochemistry

Application Code: Neurochemistry

Methodology Code: Electrochemistry
Dopamine (DA) fluctuations occur on two timescales; rapid, sub-second (phasic) firing and slower, minute-to-minute (tonic) changes. Fast-scan cyclic voltammetry (FSCV) has long been used to study phasic dopamine transmission. However, this technique requires background subtraction. Thus baseline levels, and with them tonic changes, are inaccessible using this method. A recently developed modification of FSCV, fast-scan controlled-adsorption voltammetry (FSCAV), provides access to these tonic concentrations with sub-minute temporal resolution. Here, we expand FSCAV to monitor fluctuations in tonic DA at chronically implanted carbon-fiber microelectrodes in awake animals. We measured dopamine in the dorsolateral striatum over the course of weeks. Pharmacological validation of the measured signal as dopamine was conducted using 3-methyl-p-tyrosine (AMPT) and amphetamine. These advances were then utilized to monitor the effect of sub-anesthetic ketamine injection. Sub-anesthetic ketamine has recently been investigated as a therapeutic to reduce depression, post-traumatic stress disorder (PTSD), and in our case L-DOPA-induced dyskinesia resultant from long-term treatment of Parkinson’s Disease.
Adenosine is an important neuromodulator in the central nervous system, which plays a pivotal role in a wide variety of physiological and pathophysiological processes. Previous studies have shown that tissue adenosine levels increase during ischemic events and attenuate the excitotoxic neuronal injury. Recently, our lab developed an electrochemical fast-scan cyclic voltammetry (FSCV) method using carbon-fiber microelectrodes to directly measure adenosine changes on a sub-second time scale. We found that adenosine can be transiently released and cleared in about 3 s. In this study, transient adenosine release was studied in the caudate-putamen and hippocampus of anesthetized rats during the progression of ischemia-reperfusion (I-R) injury for the first time. Transient adenosine measurements were carried out continuously for a two hour period of normoxia, followed by the induction of 30 min ischemia through bilateral common carotid artery occlusion and 90 min of reperfusion. This study suggests that transient adenosine frequency changes during early stages and immediately after the onset of ischemia and a 52% increase in number of transients was noticed during I-R compared to normoxia. Also the median inter-event time significantly decreased from 48.2 s for normoxia to 33.5 s during ischemia-reperfusion. Further, we evaluated the role of A1 and A2a receptors to mediate ischemia-induced changes in adenosine in the caudate putamen, DPCPX and SCH442416 significantly decreased the transient frequency during stroke by 38% and 21% respectively. Our study demonstrates an initial understanding on the time course release of transient adenosine changes early during ischemia-reperfusion injury.
Microdialysis is a powerful tool to investigate neural responses to drug treatments and challenges such as injuries or diseases. However, the low temporal resolution of typical microdialysis methods is a major hurdle to understand the underlying mechanisms of pathological events. We utilized improvements in high temporal resolution online microdialysis-HPLC-EC that we previously reported to study the effects of dexamethasone treatment in the striatum of rats. Evoked response to 60 mM K+, 100 mM K+, and 10 [micro]M nomifensine stimulation, as well as basal level dopamine, was measured in treated and control rats on day 1 and day 4 after dialysis probe implantation. We observed multiple distinct responses for each specific day/drug/K+ combination. Notably, dopamine levels below basal and high magnitude oscillations were consistently reproducible. Simultaneous measurement of dopamine and field potential showed strong correlation between dopamine response and field potential indicating spreading depolarization during high K+ challenge. We found that 60 mM K+ stimulation did not induce spreading depolarization and caused statistically different dopamine transients between treated and control rat in day 4, but not day 1, while 100 mM K+ stimulation induced spreading depolarization and is unaffected by dexamethasone. Pharmacokinetics of nomifensine can also be characterized from the high temporal resolution data. Our method for high temporal resolution and sensitive measurement of neurotransmitters, particularly dopamine in this case, allowed for a detailed and precise observation of evoked response, enabling study of spreading depolarization and drug pharmacokinetics with online microdialysis.
Recently, we developed a novel device called an “immuno-pillar device”, which has the desired features for POCT [1-3]. It has hydrogel pillars, fabricated inside a microchannel, with many antibody immobilized onto 1 μm diameter polystyrene beads. For the detection of disease markers, we confirmed the device provides rapid analysis with high sensitivity, it is easy-to-use, and it uses small volumes of the sample and reagents. Moreover, multiplex assay for three biomarkers was also possible. Very recently, we developed a new device, “immuno-wall device”, and a desktop reader for the immuno-wall devices, in order to apply to POCT. Using a combination of the immuno-wall device and the reader, we applied this to the tumor diagnosis during surgery [4].


Keywords: Biomedical, Detector, Immunoassay, Lab-on-a-Chip/Microfluidics
Application Code: Biomedical
Methodology Code: Microfluidics/Lab-on-a-Chip
Amperometric devices have been widely utilized for detection of cell activities, such as respiration and secretion activities, because the electrochemical detection can be performed without labeling and any damage. To achieve high-throughput analyses and imaging, several electrode-array devices have been proposed. We previously reported a large-scaled integration (LSI)-based device for bioanalysis, which was designated as Bio-LSI device. The device is based on complementary metal oxide semiconductor (CMOS) technology to incorporate 400 electrochemical sensors into the device (Fig.). In each sensor, a switched-capacitor type I-V converter is prepared to achieve highly sensitive amperometric detection. In addition, detection modes can be selected into each sensor, so that different potentials can be applied at each sensor during the amperometry. In the present study, the novel device was applied for electrochemical imaging of two kinds of target molecules at the same time. In addition, we demonstrated simultaneous detection of oxygen consumption and dopamine release from neuron-like cells (Fig.). In a conventional electrode-array device, one kind of molecules are electrochemically imaged at the same time. In contrast, the present detection system can be applied for multiplexed cell assay for simultaneous analysis, so that a lot of information of cells can be acquired. We believe that the system is useful for multi cell analyses.


Keywords: Array Detectors, Biosensors, Electrochemistry, Microelectrode
Application Code: Bioanalytical
Methodology Code: Electrochemistry
Monitorng of biomarkers such as estrogens and luteinizing hormone (LH) are important for effective production of livestock leading to profitable management. Immunosensor, which is inexpensive, available in the fields and easy to use even by farmers, is required. Therefore, we developed an electrochemical detecting platform for paper-based microfluidics as one of point-of-care testing device. This platform consists of nitrocellulose membrane, absorbent pad, electrochemical detector, and two polymethylmethacrylate (PMMA) plates. Nitrocellulose membrane was contacted with an electrochemical detector and they were sandwiched with two PMMA plates. In the electrochemical measurement, placement of working, reference and counter electrodes (WE, RE, and CE) and flow rate affect the electrochemical signal. In this study, we investigated the influences of placement of each electrodes and elapsed time after sample solution was applied on electrochemical signals.

In the first experiment, we compared two types of electrochemical detector. One electrochemical detector had WE located at upstream of RE, and the other detector had WE located at downstream of RE. Then, we conducted cyclic voltammetry and measured 10 μg/ml [i]p[/i]-aminophenol ([i]p[/i]AP) with the electrochemical detecting platform. When WE was located on downstream of RE, dissolved Ag[sup+] ion from reference electrode was detected at WE. In the second experiment, we conducted chronoamperometry and investigated the relationship between measured signal and elapsed time after [i]p[/i]AP was applied to nitrocellulose membrane. Then, we found that the electrochemical signal was inversely proportional to the sixth root of time. Thus, we have to consider these influences for quantitative immunochromatography based on electrochemical method.

Keywords: Biosensors, Electrochemistry, Lab-on-a-Chip/Microfluidics
Application Code: Agriculture
Methodology Code: Microfluidics/Lab-on-a-Chip
Nanowires recently show their great promise for analyzing cells or intracellular components due to the nanowires feature of a stealth effect against cell surfaces. Here we showed a novel usability of the nanowires to achieve an efficient capture of extracellular vesicles, which surfaces is similar to the cell surfaces, and early diagnosis based on the vesicles microRNAs. Our methodology could achieve extracellular vesicles capture in urine over 95% within 40 min, and detect cancer- and type 2 diabetes-related microRNAs from urine samples.

Keywords: Biomedical, Lab-on-a-Chip/Microfluidics, Nanotechnology
Application Code: Biomedical
Methodology Code: Microfluidics/Lab-on-a-Chip
Scanning electrochemical microscopy (SECM) is a probe microscopy technique in which an ultramicroelectrode is used as a probe. SECM is effective tool for evaluation of live cell respiration activity and neurotransmitter release. However, the resolution of the conventional SECM is micrometer scale. To improve the spatial resolution, we developed a fabrication method of nanoelectrode, the radius is less than 20 nm, based on pyrolytic carbon deposition inside glass pipet. (1,2)

Because the probe–sample distance affects the diffusion state of the molecules around the electrode, precise distance control is critical for both topography imaging and high-quality electrochemical imaging. We have developed a SECM–scanning ion conductance microscopy hybrid system, which has the dual functionality of a nanopipette and nanoelectrode, to study the topography and chemical release of living cells in a physiological environment. (1,3)

SECM-SICM is effective for high resolution chemical sensing and topographical imaging of living cells. However, the weak amperometric signal generated by the small analyte volume of these nanoelectrode is limited by the electronic capabilities of commercial current amplifiers. To overcome this weakness, we have developed a highly sensitive PPy FET on the nanometer-scaled tip. (4)

SICM nanopipette is useful to collect cytosol at the specific area on single cell for mRNA analysis. We developed a method for evaluating localization of mRNA in single cells using double-barrel SICM. (5) Two barrels in a nanopipette were filled with aqueous and organic electrolyte solutions and used for SICM and as an electrochemical syringe, respectively.


Keywords: Electrochemistry, Imaging
Application Code: Bioanalytical
Methodology Code: Surface Analysis/Imaging
Capillary electrophoresis is a powerful separation technique that is quite compatible for small-scale bioanalysis. Coupled with laser-induced fluorescence (LIF) or mass spectrometry (MS), CE provides high qualification and zmol-order detectability [1]. Although CE-LIF/MS is promising as next generation bioanalysis, some analysis such as “single cell glycomics/proteomics” still remains a challenge in terms of sensitivity. In this study, hence, technologies for further sensitivity increase in CE-LIF/MS were developed such as novel online sample preconcentration method and new sheathless electrospray ionization (ESI) emitter.

The newly developed emitter was designed from highly stable nanoESI emitter in LC-MS and high-sensitive sheathless emitter in CE-MS, so that efficient and stable ESI was obtained with no organic solvent. This emitter was employed in a CE-MS analysis of bradykinin and angiotensin II as model peptides. Analytes were well separated and detected with limit of detection of less than 1 nM (few amol).

The newly developed online sample preconcentration was designed as two-step sample preconcentration by large-volume sample stacking and transient isotachophoresis. In CE-LIF analysis of oligosaccharides, up to 2000-fold sensitivity enhancement was achieved and higher resolution than that of normal CE analysis was obtained. High salt concentration up to few mM NaCl was acceptable and obtained peaks were quite sharp. In CE-MS analysis of peptide samples such as angiotensin and bradykinin, 10 pM detectability was achieved. By coupling these two technologies, we are now proceeding single cell analysis of metabolites, peptides, proteins, and oligosaccharides, and the results would be provided in this presentation.


Keywords: Capillary Electrophoresis, Carbohydrates, Mass Spectrometry, Proteomics
Application Code: Bioanalytical
Methodology Code: Capillary Electrophoresis
Here we developed ghost cytometry, machine learning-based ultrafast fluorescence “imaging” flow cytometry, capable of high throughput (> 10,000 cells/sec) and accurate classification of different cell types, which are hard to distinguish by human eyes at any throughput.

Keywords: Bioanalytical, Biomedical, Data Analysis, Microscopy

Application Code: Biomedical

Methodology Code: Microscopy
Microfluidic droplet flows are often utilized to encapsulate sample / reagent(s) / reaction product(s) into a single droplet. For bioanalysis, aqueous droplets in organic continuous phase are used and various applications have been demonstrated. Selective enrichment of target analyte, which in one of the most important sample pretreatment methods in biochemical analysis, has not been established yet. Recently, we have investigated the selective enrichment method in the microfluidic droplets, where spontaneous emulsification on an aqueous droplet is utilized [1]. When excess amount of surfactant is added to the organic continuous phase, the surfactant micelles are formed in the organic phase. When the micelles approach to the aqueous droplet interface, hydrophilic space inside the micelles is hydrated by water transferred from the microdroplet through organic membrane. After acceptance of water, the micelle becomes 100-nm-sized aqueous droplet (nanodroplet) as shown. Then, the microdroplets shrink. During the water transfer, solutes inside the microdroplets remain inside it, or be partitioned to the nanodroplets with the water transfer depending on its hydrophobicity and/or size of the species. In the presentation, the partition/concentration trend dependent on the solute type will be discussed.


Keywords: Bioanalytical, Extraction, Fluorescence
Application Code: Bioanalytical
Methodology Code: Microfluidics/Lab-on-a-Chip
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<td>Centrifugal Microfluidic Device with On-Board Reagents and Smartphone Colorimetric Detection for Explosives Identification</td>
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| Primary Author| Shannon T. Krauss  
University of Virginia |
| Co-Author(s)  | Brian E. Root, James P. Landers, Victoria C. Holt |
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| Room:         | W476 |

**Abstract Text**

Colorimetric reactions have been extensively used for on-site explosives analysis as a rapid and inexpensive detection method. 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. Here, we describe a centrifugal microfluidic system that accepts single-use, disposable microchips that, with embedded reagents, cost’ <$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 explosive or explosive group. On-board reagent storage was explored by utilizing a novel polyester-paper based hybrid device fabrication method and evaluated for color stability, with no loss in effectiveness of the reagents for color change observed over 10 weeks. Additionally, where the color is known to have a short-term stability in solution (30 sec), paper allowed stability for a minimum of 24 hours, representing ~3000-fold improvement. The proof of principle prototype system (microdevice, instrument and smartphone) was used for multiplexed testing for the presence of various explosive materials from a single input, such as TNT, urea nitrate, and ammonium nitrate. Color analysis was used to determine quantitative hue and saturation values associated with positive results for each explosive. These thresholds were applied to a custom-built cellphone app for user-friendly analysis.

**Keywords:** Automation, Detection, Identification, Lab-on-a-Chip/Microfluidics  
**Application Code:** Homeland Security/Forensics  
**Methodology Code:** Microfluidics/Lab-on-a-Chip
Accuracy and reliability of data generated by the HAPSITE ER (Hazardous Pollutant On-Site Extended Range) was improved by modifying thermal desorption (TD) tubes prior to field sampling. In the past, HAPSITEs have quantitative data variances of >50% relative standard deviation (RSD) on the same instrument and even greater RSD when comparing multiple instruments.

Recently, we determined that the addition of focusing agents, similar to the compounds of interest, spiked onto TD tubes prior to sampling can achieve a standard deviation of <15% (Rubenstein et al; Harshman et al). Here we established three-point calibration curves by adding 3 distinct masses of a singular analyte to TD tubes. We investigated stable isotopically labeled compounds (ILCs) diethyl malonate (MW 160), diethyl malonate-2-13C (MW 161), and diethyl malonate-1,2,3-13C (MW 163). Stability of the study was examined over 22 days by spiking TD tubes each week and compared to tubes prepared at the beginning of the experiment.

Data accumulated over 22 days will be discussed. R² values remained constant at 1± 0.002 when graphing area vs. concentration indicating that the trends are linear and are appropriate as calibration curves. The data was also normalized through the process of dividing area by the on column concentration. From the normalized data RSD values were calculated at 2.687% on the same instrument and 9.827% between instruments.

Calibration curves based on ILCs should be useful not only for the unlabeled compound; matching the relative response to nerve agents should improve the field measurement of nerve agents. Work in progress will be presented to support this. If successful, this could eliminate the need to send confirmatory samples back to a reference laboratory and provide near real-time accurate data.

Keywords: Gas Chromatography/Mass Spectrometry, Organic Mass Spectrometry, Semi-Volatiles, Thermal Desorption
Application Code: Homeland Security/Forensics
Methodology Code: Mass Spectrometry
Analysis of Explosives and Chemical Weapons for Forensics Applications (Half Session)

Real-Time Mass Spectrometry Detection of Remotely Sampled Vapors and Aerosols by Venturi-Assisted Entrainment and Ionization

The recent evolution of homemade explosives, notably high volatility peroxide-based and nitrate ester explosives, has reinforced the need to efficiently collect, transport, and detect vapors and aerosols in real-time. Advancements in vapor collection and detection platforms to complement the traditional manual swab-collection detection technologies, remain imperative to the defense, homeland security, customs and border protection, and forensic science sectors. Here, the Venturi-assisted ENTrainment and Ionization (VENTI) system was developed, integrating the Venturi and Coanda effects to establish efficient collection and transport of remotely sampled vapors, aerosols, and dust particulate for ambient ionization mass spectrometry (MS) detection. The VENTI system requires no moving parts or vacuum pumps and uses robust air amplifier components for flow control in conjunction with atmospheric pressure chemical ionization (APCI). Remote sampling probes/lines up to 2.5 m long, as well as a continuous real-time facility monitoring configuration were investigated. Venturi-assisted entrainment exhibited enhanced aerodynamic reach and collection over simple suction for vapor detection, which was corroborated with laser-light sheet visualization and schlieren imaging. Detection of vapors, aerosols, and dust particulate from volatile chemicals, explosives (peroxide-based, nitrate ester, and nitroaromatic), narcotics, and chemical warfare agent (CWA) surrogates was demonstrated. The VENTI platform, with its many envisioned configurations, may play an impactful and complementary role to the traditional swab-based trace residue and particulate collection and detection systems.

The U.S. Department of Homeland Security Science and Technology Directorate sponsored a portion of this work.

Aerosols/Particulates, Detection, Forensic Chemistry, Mass Spectrometry

Homeland Security/Forensics

Mass Spectrometry
Standard spectroscopic approaches to chemical sensing require the collection of well-defined transmission, absorption, or reflection spectra of the chemical(s) of interest. These spectra are then compared to known references found in a spectral library, using one of several numerical methods, to identify the chemical. We have recently developed a non-spectroscopic approach to chemical sensing that utilizes multiple, broadband, overlapping infrared (IR) optical filters to discriminate between chemicals with similar IR absorption features. This biomimetic approach, based on human color vision, operates by generating a unique detection vector defined by the interaction between the IR absorption bands of a chemical to a set of three, broadband, overlapping IR optical filters. A gas-phase sensor operating in the mid-IR, which utilizes this biomimetic approach to identify chemicals of interest has recently been designed and assembled.

We present an overview of the design and performance of this sensor, and demonstrate its ability to detect and discriminate between chemicals with similar mid-IR absorption bands. High-confidence discrimination is observed using this sensor, with a low number of false positives. We present the results of experiments with multiple chemicals with similar mid-IR absorption signatures, and discuss future development and application of this non-spectroscopic approach for chemical detection.

Keywords: Chemometrics, Identification, Infrared and Raman, Sensors
Application Code: Homeland Security/Forensics
Methodology Code: Sensors
### Abstract Title

**Sexual Offender Nodal Isolation of Cells (SONIC): Acoustophoretic Separation of Sperm Cells from Mock Sexual Assault Samples**

### Abstract Text

Tens of thousands of sexual assault kits (SAKs) sent to crime labs in the United States remain stored and untested for more than 30 days. This backlog is largely due to time-consuming, labor- and instrument-intensive processing of SAK samples for DNA analysis. Based on pioneering work from the Laurell and Nilsson groups, we aim to exploit acoustic microfluidics to enhance SAK analysis by expediting the first step in analysis – differential extraction (DE) of the sperm cells from the female epithelial cells.

We report acousto-isolation of sperm cells from mock sexual assault samples with a completely revamped microchip design that allows for automated frequency scanning, trapping and physical separation of the male (sperm) and female (epithelial cells) components driven by a custom software. The effectiveness of the sperm cell isolation was determined by evaluation of DNA in the female and male fractions using STR profiling, with clean male DNA profiles obtained from samples with female:male cell ratios as high as 20:1. Sample separation occurs in the trapping region of our device, where sound frequencies between 7-9MHz generate three low pressure trapping nodes. By tuning the frequency of the sound waves, and thus the size and location of the nodes, particles can be selectively retained in the channel.

Our current work is optimizing high cell-count samples and testing post-coital vaginal swabs. The SONIC system will have an immediate impact on the forensic field, as improvements in efficiency and throughput of SAKs will result in faster resolution of sexual assault investigations.

### Keywords

- Biotechnology
- Forensics
- Identification
- Lab-on-a-Chip/Microfluidics

### Application Code

- Homeland Security/Forensics

### Methodology Code

- Separation Sciences
Developments in Forensics and Homeland Security Analyses (Half Session)

Chirp Delay Heterodyne Infrared Spectroscopy with Pulsed Distributed Feedback Quantum Cascade Lasers

This presentation introduces a new infrared spectroscopic platform for signal amplification relying on the generation of a heterodyne modulation with pulsed distributed feedback quantum cascade laser (DFB-QCLs).

We first present the technique, which uses the natural chirp of DFB-QCLs to generate a heterodyne beat between two portions of the laser beam (reference and signal), one delayed with respect to the other. No frequency-shift or phase-modulation device is needed and the time delay between signal and reference controls the frequency of the heterodyne modulation of the measured intensity. Different data analysis strategies are discussed to optimally retrieve the signal intensity from the amplitude of the MHz-range modulation. The technique relies on the extremely stable center frequency and frequency chirp profile of pulsed DFB-QCLs.

Experimental results are presented demonstrating signal retrieval for strongly absorptive samples (Absorbance>4) and 1-meter distance standoff diffuse reflectance, with two order of magnitude signal enhancement. Polarization and speckle effects originating from the roughness of the analyte are discussed.

The demonstrated signal amplification paves the way for faster standoff chemical analysis of residues and remote gas phase detection. Such speed increase can be leveraged for scanning-type hyperspectral imaging for high throughput hazard localization.

Keywords: Infrared and Raman, Laser, Spectroscopy, Vibrational Spectroscopy
Application Code: Homeland Security/Forensics
Methodology Code: Vibrational Spectroscopy
Ethanol Concentration in 63 Refillable Electronic Cigarettes Liquid Formulations Determined by Headspace Gas Chromatography with Flame Ionization Detector (HS-GC-FID)

Personal battery powered vaporizers or electronic cigarettes (e-cigarettes) were developed as an alternative to traditional cigarettes. In May 2016, the US Food and Drug Administration (FDA) imposed regulatory statutes on e-cigarettes and their liquid formulations (e-liquids), prior to then they were unregulated. E-liquids are typically composed of propylene glycol and/or glycerin, flavoring component(s), and active ingredient(s), such as nicotine. Sixty-three commercially available e-liquids were purchased from various sources. These e-liquids contain a variety of flavors and active ingredients. None of these e-liquids listed ethanol as a component.

A headspace gas chromatography with flame ionization detector (HS-GC-FID method routinely employed for the quantification of volatiles in forensic and clinical specimens was used to analyze these e-liquids. The chromatographic separation of the volatiles was performed on a Restek BAC-1 column. A linear calibration was generated for ethanol with limits of detection and quantification (LOD/LOQ) of 0.005 % by weight by volume. Ethanol concentrations in the e-liquids ranged from none detected to 19 % by weight by volume.

The ethanol present in these products may have been a flavorant or a solvent; the reason for inclusion cannot be fully ascertained. The implications of vaporizing ethanol as an e-liquid component are unknown.

This project was supported by U.S. Department of Justice Award No. 2014-R2-CX-K010, and the National Institutes of Health Award No P30DA033934. The opinions, findings, and conclusions or recommendations expressed in this publication/program/exhibition are those of the author(s) and do not necessarily reflect those of the Department of Justice.

Keywords: Analysis, Forensic Chemistry, Gas Chromatography
Application Code: Homeland Security/Forensics
Methodology Code: Gas Chromatography
Rare earth elements (REE) and certain alkaline earths can produce \([\text{M}^+2]\) interferences in ICP-MS because they have sufficiently low second ionization energies. Four REEs (\(^{150}\text{Sm}\), \(^{150}\text{Nd}\), \(^{156}\text{Gd}\) and \(^{156}\text{Dy}\)) produce false positives on \(^{75}\text{As}\) and \(^{78}\text{Se}\) and \(^{132}\text{Ba}\) can produce a false positive on \(^{66}\text{Zn}\). Currently, US EPA Method 200.8 does not address these as sources of false positives. Additionally, these \([\text{M}^+2]\) false positives are typically enhanced if collision cell technology is utilized to reduce polyatomic interferences associated with ICP-MS detection. Correction equations can be formulated using either a unit or \(\frac{1}{2}\) mass approach. The \(\frac{1}{2}\) mass correction approach does not suffer from the bias generated from polyatomic or end user based contamination at the unit mass but is limited by the abundance sensitivity of the adjacent mass. For instance, the use of \([\text{m/z} 78]\) in a unit mass correction of \([\text{m/z} 156]\) \([\text{m/z} 78]\) can be biased by residual \([\text{m/z} 40]_2\text{Ar}\) while the \(\frac{1}{2}\) mass approach can use 77.5 or 78.5 and is limited by the abundance sensitivity issues from mass 77 and 78 or 78 and 79, respectively. This presentation will evaluate the use of both unit and \(\frac{1}{2}\) mass correction approaches as a means of addressing \([\text{M}^+2]\) false positives within the context of updating US EPA Method 200.8. This evaluation will include the analysis of As and Se standards near the detection limit in the presence of low (2ppb) and high (50ppb) levels of REE with benchmark concentrations estimated using triple quadrupole ICP-MS.
It is challenging to separate and measure the physical and chemical properties of monometallic and bimetallic engineered nanoparticles (NPs), especially when mixtures of NPs consist of particles of similar size, composition, and especially when present at low concentrations. One of the most difficult properties to routinely measure in a NP suspension is particle number concentration, which can be measured directly using techniques like nanoparticle tracking analysis (NTA) or by a complex, multimethod approach. However, these techniques have limitations when dealing with mixtures of NPs, as they do not offer compositional information. As a standalone technique, Single Particle ICP-MS (SP-ICP-MS) has emerged as the technique of choice for the detection of metallic NPs at environmental levels, providing particle size, size distribution, and particle number concentration all on particle per particle basis. In this work, we demonstrate the use of SP-ICP-MS as a stand-alone technique to measure complex suspensions of NPs and discriminate between mixtures of monometallic gold (Au) and silver (Ag) NPs and bimetallic Au@Ag (core-shell) NPs, yielding particle-size and particle-number concentrations for each constituent.

**Keywords:** Biological Samples, Characterization, ICP-MS, Nanotechnology

**Application Code:** Nanotechnology

**Methodology Code:** Atomic Spectroscopy/Elemental Analysis
Engineered nanoparticles (ENPs) are increasingly used in many fields since they exhibit unique properties that cannot be found in bulk-sized materials, which has been reported to lead to potential adverse effects on the environment and human health. Heavy metals are well known toxic pollutants. However, the synergistic toxic effect of ENPs and heavy metals on cells were not well investigated. In this study, a novel single cell inductively coupled plasma – mass spectrometry (SC-ICP-MS) method for rapid monitoring of ENP and heavy metal uptake by yeast cells was developed. Yeast (Saccharomyces cerevisiae) cell line was selected as a model system and cultured by following the standard procedure. The diluted cell suspension and heavy metal element solutions were introduced to a NexION 350D ICP-MS with Syngistix Nano Application module for SC-ICP-MS analysis. The amount of ENPs and heavy metals uptake/associated with yeast cells can be quantitatively determined. Detailed experimental procedures and results will be presented at the conference. This novel, rapid and sensitive method will pave a new route for investigation of ENP and heavy metal toxicity at the single cell level.

Keywords: ICP-MS, Metals
Application Code: Bioanalytical
Methodology Code: Atomic Spectroscopy/Elemental Analysis
ICP-MS as an Universal Tool (Half Session)

The Preparation and Analysis of Mineral Based Excipients for ICH Q3D/USP <232>
Elemental Impurities by ICP-MS

To date excipients have typically not been routinely tested for elemental impurities using methods capable of specific and sensitive detection; therefore, current metal concentrations and variability is unknown. In particular many metals are naturally present in mined excipients and cannot be removed through processing. Natural variation can be expected in the material sources and, therefore with the implementation of USP chapter 232 and ICH Q3D, pharmaceutical manufacturers will need to develop an understanding of the risk of unwanted metals being present in their products.

We have developed ICP-MS methodology capable of determining low levels of elemental impurities in excipients and present data on a range of mined excipients for which monitoring will be required by pharmaceutical manufacturers or excipient suppliers under UPS <232> and ICH Q3D.

| Keywords: | Elemental Analysis, Elemental Mass Spec, ICP-MS, Pharmaceutical |
| Application Code: | Pharmaceutical |
| Methodology Code: | Atomic Spectroscopy/Elemental Analysis |
Considerations for Quantitative Method Transfer Across Chromatographic Systems

Transfer of reversed phase liquid chromatography (LC) methods across both HPLC and UHPLC chromatographic instrumentation requires careful consideration of the operating parameters and design of each instrument. For example, gradient formation can be influenced by dwell volume, while linearity and quantitation can be affected by the mechanism for sample injection, as well as the detector. Lastly, thermostating can alter retention—whether due to frictional heating affects, mobile phase pre-heating or thermal gradients. To understand the effect these factors may have on methods transfer, both instrument characteristics and specific method conditions must be factored and evaluated when transferring HPLC methods.

In this presentation a quantitative LC method will be analyzed across HPLC and UHPLC systems. Testing will be conducted to measure specific characteristics of the system, and demonstrate how these characteristics can impact methods transfer. Comparison of different instrument configurations, including the impact of the method options available on each system, will also be performed. For example, detector settings and needle wash options will be evaluated for their impact on methods transfer. System suitability criteria will be used to evaluate the separations. Based on the effects of individual system characteristics, strategies for successful methods transfer will be described. In these examples, consideration will be made to conduct method transfer in accordance with regulatory guidelines for allowable adjustments to compendial methods.

Keywords: Liquid Chromatography, Liquid Chromatography/Mass Spectroscopy, 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 by transiently cooling the head of the column using high powered thermoelectric coolers. In TASF, the head of the column must change temperature quickly and analyte retention must respond to column temperature changes. TASF was modelled using COMSOL Multiphysics to predict maximum flow and column diameter. These simulations showed that capillary and 1 mm microbore columns can be used with TASF. TASF is most effective for solutes with large retention enthalpies. When the pH of the mobile phase is close to the pKa of the analyte, buffer ionization enthalpy significantly contributes to the overall adsorption enthalpy, which consists of analyte adsorption, analyte ionization, and buffer ionization enthalpies. By choosing buffers with different enthalpies of ionization, the overall adsorption enthalpy and response of chromatographic retention to temperature can be optimized for TASF. High performance liquid chromatography (HPLC) has been used to study retention factors and overall adsorption enthalpy in the presence of different buffers, however individual reaction enthalpies cannot be measured using HPLC. Adsorption and ionization enthalpies were studied using isothermal titration calorimetry with buffer ionization enthalpies ranging between -7 kJ/mol and 42 kJ/mol. Dissociation constants were measured using potentiometric titration methods in mobile phase (80:20, Water:Acetonitrile). A program was written to predict observed overall adsorption enthalpy by HPLC using individual thermodynamic parameters of retention and ionization. This allows for the optimization of TASF for ionizable analytes through buffer selection.
Convolution Approach to Speed Up Simulation for Various Conditions of Liquid Chromatography Including Volume Overload and Solvent Mismatch

Liquid chromatography (LC) simulators are effective method development tools. Our simple simulation tool based on the Craig distribution model can predict retention times and peak variances that are in good agreement with closed form theory. Additionally, it accurately simulates more complex gradient shapes (i.e., nonlinear) and conditions involving sample/eluent solvent mismatch and injection volume overload that are prevalent in two-dimensional liquid chromatography (2D-LC). Often simulation of such complex LC conditions requires increased computation time. One possible way to get around this problem is to consider two distinct parts of the separation: 1) a short segment of the column inlet where the peak shape changes rapidly as a function of the sample input and initial elution conditions; and 2) a longer segment of the column where the peak shape is stable, and simply changes in width and height. The former part of the separation must be predicted by numerical simulation while the latter can be predicted using closed-form theory, which is much faster than numerical simulation. The predictions from these two parts are then convolved to give the predicted retention times and peak variances for the total separation. This approach can be extended to the second dimension separation in 2D-LC, where the first dimension effluent is often diluted to minimize solvent mismatch between sample solvent and second dimension mobile phase. It is desirable to be able to simulate these conditions to enable optimization of 2D-LC methods. This approach will be validated against experimental and full simulation results.

Acknowledgements: This research is supported via grants from NSF (CHE-1507332; CHE-1609449). LNJ is supported by an Altria Graduate Student Fellowship.

Keywords: Computers, HPLC, Liquid Chromatography, Software
Application Code: General Interest
Methodology Code: Liquid Chromatography
The analysis of amino acids (AAA) is an essential tool for many fields of research and is a required measurement in many product related applications. The number of analytes to be measured in a single determination ranges from the twenty species that make up proteins to the forty or fifty commonly monitored as metabolic markers. AAA is a particularly challenging analytical problem. The range of compounds covers a broad spectrum of chemical properties while including similar and even isomeric structures. A high resolution separation technique is required. The assay is complicated by the absence of a common chromophore or convenient detection functionality. These challenges resulted in two major approaches to AAA. Classically, the separation used ion exchange chromatography followed by post-column reaction detection. The analysis has also been commonly performed using a pre-separation derivatization of the amino acids, followed by reversed-phase chromatography. This latter alternative adds detectability while giving improved separation. Both approaches can yield accurate and precise results, but they both have significant operational disadvantages. In the current study, alternative methods are developed and compared to the traditional methods. The separations are based on either high retention reversed-phase with ion suppression, or ion-pairing with volatile reagents, or HILIC chromatography. These separations all eliminate the derivatization step, but none provides complete chromatographic resolution of all the possible combinations of amino acids. Each of the chromatographic techniques uses detection with electrospray mass spectrometry for universal detection. Mass spectrometry also provides information-rich selective detection, reducing the need for complete chromatographic resolution. The new methods are compared to established techniques for protein hydrolysates, cell culture media, and hydrolyzed food products.

Keywords: Amino Acids, Biopharmaceutical, Liquid Chromatography, Liquid Chromatography/Mass Spectroscopy
Application Code: General Interest
Methodology Code: Liquid Chromatography
Fabrication of Fused-Silica Capillary Columns for On-Column UV-Absorption Detection in Capillary Liquid Chromatography

Liquid chromatography with UV-absorption detection (HPLC-UV) is an established, powerful analytical technique for analysis of complex nonvolatile samples. The UV-absorption detector is ideal for routine applications. However, UV-absorption detection has limited ability to differentiate compounds; therefore, retention data are extremely important for identification. As a result, good resolution and high peak capacity are critical to separate and positively identify target analytes. On-column detection when using small diameter capillary columns improves the chromatographic resolution by reducing post-column band broadening. In this work, fused-silica capillary LC columns with UV-transparent window and near-zero post-column dead volume were fabricated by forming short organic monolithic plugs at both ends of the packed LC column to contain the packed bed. Various stationary phase particles (1.7 and 3 µm, C18 and biphenyl) were packed inside 150 µm i.d. UV transparent capillaries before the monolithic frits were added to both ends. The resultant columns were connected to a custom-built nano-flow LC system with light-emitting diode (LED) UV-absorption detector and demonstrated for positive identification and quantitation of target compounds.

Keywords: Capillary LC, Chromatography, HPLC, HPLC Detection
Application Code: General Interest
Methodology Code: Liquid Chromatography
A robust and customizable stationary phase is desired to enhance the separation and sensitivity of the on-line monitoring of neurotransmitters using HPLC. Hyper-crosslinked polymer coated stationary phases have previously been made using a long, tedious procedure involving metal catalysts that can be detrimental to chromatography. As a vastly simpler, metal-free alternative, we synthesized polymer coated stationary phases using the thiol-yne reaction. The thiol-yne reaction is a widely used click chemistry reaction that can create highly cross-linked polymers without catalysts that harm chromatographic performance. To create a reversed phase 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 then cross-linked using 1,6-hexanediithiol and a small amount of DEB. A mixed mode strong cation exchange (SCX) stationary phase was also made by the addition of sodium 3-mercaptop-1-propanesulfonate as a reagent during the crosslinking step. The reversed phase material shows the expected hydrophobic characteristics and gives good, symmetrical peak shapes for model solutes. The SCX stationary phase has a zeta potential at pH 2 of -19 mV. Compared to the bare silica’s zeta potential of 0 mV, it is apparent that a negative charge has been added to the surface. The thiol-yne reaction can produce a crosslinked polymer coating that allows customized stationary phases. This crosslinked coating should increase the stationary phase’s stability and durability.

Keywords: HPLC, HPLC Columns, Liquid Chromatography, Modified Silica
Application Code: General Interest
Methodology Code: Liquid Chromatography
Since its introduction in the 1990s, hydrophilic interaction liquid chromatography (HILIC) has become increasingly popular due to its capability for separation of complex polar compound mixtures and its compatibility with mass spectrometry (MS) detection. The addition of a third solvent to the mobile phase of acetonitrile (MeCN) and ammonium acetate buffer has proven effective at separating aromatic acid geometric isomers. Phenolic acids are two classes of compounds commonly found in fruit juices, wines, and beers. Many liquid chromatography (LC) methods have been developed to detect, separate, and quantitate these acids; however even though these methods are quick, they require complex mobile phase gradients. The aim of the project is to develop a simple, fast, and isocratic LC-MS method to separate many of the phenolic acids found in beverages. A plain silica column (150 x 4.6 mm, 3 [micro]m) and a flow rate of 0.4 mL/min were used. The mobile phase consisted of MeCN/ammonium acetate, 10 mM, pH 6/pentane (90/5/5) and detection was at 254 nm. Near baseline separation of ten phenolic acids (2-hydroxybenzoic, 3-hydroxybenzoic, 4-hydroxybenzoic, 2-hydroxycinnamic, 3-hydroxycinnamic, 4-hydroxycinnamic, syringic, vanillic, ferulic, and sinapic acids) was achieved in under 40 minutes. Two acids were difficult to separate, however since they are not isomers, MS should be able to distinguish the presence of both acids. Analytical figures of merit, including limit of detection and limit of quantitation, have been determined.

Keywords: HPLC, Liquid Chromatography/Mass Spectroscopy
Application Code: Food Science
Methodology Code: Liquid Chromatography
While fermentation as it relates to beer brewing has been exploited for thousands of years, methods of quantitatively evaluating beers and ales before, during, and after fermentation are only now becoming widely available.

The three aspects of brewing which are suited to monitoring are the sugars involved. Monitoring of the sugars involved is an assay of a complex enzymatic system, one whose products are due to the activity of a number of enzymes. Each of the enzymes in the system has different optimum conditions under which it operates. By altering the conditions, the activity of the enzymes changes. As a result, the amounts of sugars produced and their proportions will change, and this in turn will alter the resulting beer’s taste.

Likewise, the concentration of free amino acids in beers depends on various factors; amino acids are the building blocks of proteins in the raw materials used for beer production, they influence the activity of cereal proteolytic enzymes in the course of various mashing procedures, as well as the microorganisms involved in the fermentation process activity.

While many larger brewing companies have had substantial laboratory support to help with these analyses, smaller companies have not. Many craft beer producers are using different grains, with untried combinations of yeasts to produce unique and tasty beverages. Understanding how these combinations work could help them optimize their processes for taste as well as yield.

In this work, amino acids and sugar content are measured throughout the beer making process, from malting to finished product. The amino acids are measured using OPA/FMOC derivatization on high-pH stable superficially porous InfinityLab Poroshell HPH C18 columns. Sugars and alcohol are measured before during and after the fermentation process using Hi-Plex ligand-exchange columns.

Keywords: Amino Acids, Beverage, Carbohydrates, Chromatography
Application Code: Food Science
Methodology Code: Liquid Chromatography
Hybrid plasmonic colloidal nanoparticles and their interactions with biological molecules, dye molecules, and light are investigated using nonlinear and ultrafast spectroscopy. The photocleaving dynamics of microRNA-functionalized silver nanoparticles in colloidal suspension in water are monitored using time-dependent second harmonic generation (SHG) measurements. MicroRNA is functionalized to 65 nm spherical colloidal silver nanoparticles using a nitrobenzyl linker that cleaves upon ultraviolet irradiation. The real-time SHG measurements determine the photocleaving rates under varying laser irradiation wavelengths and powers. The results are compared to corresponding SHG measurements in microRNA-functionalized polystyrene nanoparticles, showing that the silver nanoparticles display a six-fold plasmon enhancement in photocleaving with a maximum efficiency at 365 nm. Additionally, molecular adsorption, resonant coupling, fluorescence enhancement and quenching, and altered ultrafast excited-state dynamics are studied for dye molecules interacting with colloidal gold-silver-gold core-shell-shell nanoparticles. SHG is used to determine the adsorbate site density and free energy of adsorption for brilliant green and rhodamine 110 adsorbing to plasmonic nanoparticles in water. Significant energy transfer and faster excited-state lifetimes are observed for rhodamine 110 while negligible energy transfer is observed for brilliant green from transient absorption spectroscopy measurements. Finally, photothermal cleaving dynamics are determined using time-dependent SHG measurements under near-infrared irradiation in microRNA-functionalized gold-silver-gold core-shell-shell nanoparticles using a thermally-activated linker. These nonlinear and ultrafast spectroscopic investigations provide important information on the surface chemistry, photocleaving rates, and enhanced molecular sensing in hybrid plasmonic nanoparticles for potential biological applications.
We report direct evidence for long range (ca. 100 micrometer) order in the room temperature ionic liquid (IL) 1-butyl-3-methylimidazolium tetrafluoroborate (BMIM+BF4-), supported on a silica surface. We have measured the rotational diffusion dynamics of anionic, cationic and neutral chromophores as a function of distance from the silica support. The results reflect the excess charge density gradient induced in the IL by the charge present on the silica surface. Identical measurements in ethylene glycol reveal spatially invariant reorientation dynamics for all chromophores. Capping the silica support with Me2SiCl2 results in spatially invariant reorientation dynamics in the IL. We understand these data in the context of the IL exhibiting a spatially-damped piezoelectric response mediated by IL fluidity and disorder.

There are two immediate implications of the findings reported here. The first is that by devising a means of characterizing free charge gradient in ILs, we have demonstrated for the first time a level of organization that exceeds previously reported gradients in this family of materials. The ability to relate this gradient to applied surface charge brings with it the possibility of controlling material properties such as refractive index gradients in ILs. Perhaps of more fundamental value is that this family of materials can be understood in the context of piezoelectric materials where the bulk properties damp the spatial extent of the potential gradient owing to the disorder intrinsic to any liquid phase molecular system. Evaluating the dependence of free charge gradient on the identity of the IL will help to determine the range of utility for this effect.

Keywords: Fluorescence, Molecular Spectroscopy
Application Code: General Interest
Methodology Code: Molecular Spectroscopy
Plasmon waveguide Raman spectroscopy using a scanning angle (SA) format is applied to study polymer films and monolayers. The technique is capable of simultaneously measuring polymer thickness, chemical composition, structure and locating buried interfaces with tens of nanometer spatial resolution in the axial direction; and it has the sensitivity for measuring monolayers at smooth gold films. The technique uses a prism with a thin noble metal layer on which the polymer or monolayer is deposited. Raman spectra are collected as the incident angle of the excitation laser is precisely varied from 30 to 70 degrees. SA Raman spectra of 10 nm to 2 micron polystyrene, polycarbonate or block copolymer films are collected with second acquisition times and signal-to-noise ratios that exceed 100. Unlike other analytical techniques that can provide thin film thickness measurements, the chemical content inherently encoded in the Raman signal allows the index of refraction, film compositions, and chemically-distinct interfaces to be concurrently measured along with the thickness. Finally, the sensitivity of the measurement enables monolayers to be measured with a signal that is both highly reproducible and calculable. SA Raman spectroscopy is a versatile method applicable whenever the chemical composition, structure and thickness of interfacial thin films needs to be measured with high axial resolution, and when monolayer sensitivity is need on smooth metal films.

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.
Polarizability is a measure of the degree to which charge is distorted within a molecule or atom in response to an external electric field. Typically, the experimental polarizability is determined by accurate measurements of a dielectric constant.\cite{1} With terahertz time-domain spectroscopy (THz-TDS), frequency dependent dielectric properties can be obtained directly by analysis of differences in phase and amplitude between THz time-domain signals collected for air and the sample of the interest.\cite{2} The low energies associated with terahertz radiation enables probing inter-molecular interactions.

In our research, THz polarizability is being explored as an analytical tool to characterize molecule-molecule interactions within assemblies of organic cocrystals. Polarizability values are determined for a set of organic crystals and co-crystals across 0.3-3.0 THz optical frequencies by using a combination of effective medium theory\cite{3} and the Clausius-Mossotti relationship.\cite{4} Polarizability measurements will be presented for salicylic acid and acetylsalicylic acid, well known active pharmaceutical agents (API), as well as for a set of cocrystal formers (CCFs), including 4, 4'-bipyridine, 1, 2, bis (4-pyridyl) ethane, 1, 2-di (4-pyridyl) ethylene, and 1, 2-di (4-pyridyl) propane and those of the corresponding cocrystals in form of API\textsuperscript{CCF}API. The measured THz polarizabilities were compared with values predicated from an additive atomic polarizability model. Result indicate that a simple additive model works well to predict polarizability for single components but not for cocrystal assemblies. A more sophisticated polarizability prediction model is required to consider the complex intermolecular interactions associated with cocrystal systems.

Reference:

Keywords: Materials Characterization, Method Development, Molecular Spectroscopy, Pharmaceutical
Application Code: Pharmaceutical
Methodology Code: Molecular Spectroscopy
Small-scale laboratory mills are commonly used in an attempt to predict the performance of scaled up experimental milling that approaches commercial operation. Various small-scale laboratory mills are used to evaluate either small quantities of early generation wheat cultivars or to analyze blends or choice of a particular variety before purchase by a major milling company. Wheat milling has long been considered an art and requires balance among many different individual unit processes. However, the laboratory experimental milling does not necessarily mimic the large scale process. The use of near infrared quantitative chemical imaging that is sensitive to small differences is especially useful for this study because it is not dependent upon the particular wheat cultivar, the growing location, or the mill being used. This method has a chemical basis that enables direct measurement of the endosperm content of the flour, whereas traditional methods report the impurity present with the endosperm as minerals or off color from the outer layers of the kernel. Unfortunately, the age old methods produce different results for different wheats. Flour and milling byproduct streams are compared for various laboratory milling systems in this study, with the results of a pilot scale milling process applied to the same wheat blend.

Keywords: Imaging, Quality, Quantitative, Spectroscopy
Application Code: Quality/QA/QC
Methodology Code: Vibrational Spectroscopy
Lectins are a broad class of carbohydrate-binding proteins which are involved in a variety of biological processes including cell-adhesion, immune response and apoptosis. Concanavalin A (ConA) is one such protein which binds selectively to mannose groups and has received considerable interest because of its use in protein characterization on cell surfaces, agglutination of oncogenic cells, impact on expression of cell-surface enzymes, and ability to induce T cell mitosis. Various approaches have been taken to investigate lectin-carbohydrate interactions, including sandwich assays, light scattering measurements, or surface-plasmon resonance in polymer particles or on gold surfaces. These methods for measuring lectin-carbohydrate interaction struggle to meet the challenges of rapid, structurally specific detection from low-concentration samples. Here we describe the preparation and use of supported phospholipid bilayers deposited in high surface area chromatographic silica particles as a platform for confocal Raman microscopy-based detection of the specific binding of ConA to mannose-functionalized phospholipids. Given the high specific surface area of the support, lipid bilayers formed within a chromatographic particle allow for label free detection of the specific ConA-mannose association within a single ~60 femtoliter chromatographic particle from low micromolar protein solutions. Additionally, Raman detection allows for protein-specific detection and is sensitive to changes in bilayer structure and can be carried out rapidly from low (microliter) sample volumes. By varying the fraction of mannose-functionalized lipid in the bilayer preparation, accumulated ConA population within the particle can be controlled.

Keywords: Biosensors, Lipids, Microspectroscopy, Raman Spectroscopy
Application Code: Bioanalytical
Methodology Code: Vibrational Spectroscopy
Chemical composition of foreign particles found in therapeutic formulations should be identified in order to determine and eliminate their sources. Traditionally, particles are isolated for such analyses. However, isolation of particles can lead to cross contamination and loss of particles. Therefore there is also a need of in situ analysis of particulate matter.

In this work we will give an overview of a rapid sample screening to determine number and size of particles, followed by compositional analysis. We will discuss the importance of direct correlation of the particles with their potential sources in addition to determining chemical composition. We will also demonstrate some recent success in particulate analysis directly within a container via Raman spectroscopy. This method allows quick root cause investigation of extrinsic and intrinsic particles and helps to prevent further contamination in production.

Left and middle sections of the Figure below show spectra of extrinsic visible particles of polyethylene and cellulose analyzed inside of a 1 ml glass syringe with 1 mm thick walls. The right section of the Figure shows the spectrum of a stain discovered inside of a 10 ml vial. The spectrum shown here was collected by focusing the laser beam through the bottom of the intact vial on the inside, where the stain was located.

This work demonstrates the importance of rapid particle analysis and source identification in support of root cause investigations. In situ analysis helps to avoid time-consuming sample preparation that can lead to the potential loss of particles and cross contamination.

Keywords: Molecular Spectroscopy, Pharmaceutical, Quality Control, Raman Spectroscopy
Application Code: Quality/QA/QC
Methodology Code: Vibrational Spectroscopy
Infrared p-polarized multiple-angle incidence resolution spectrometry (IR pMAIRS) is a recently developed powerful tool to reveal the molecular orientation in a thin film deposited on an IR transparent substrate made of such as silicon, germanium and calcium fluoride. IR pMAIRS works powerfully for a thin film of most organic materials having a refractive index of ca. 1.55. However, some exceptional materials exhibit an apparently high or low refractive index, typically found for fullerene and perfluoroalkyl compounds, which makes pMAIRS analysis inaccurate. In the present study, this problem has readily been removed by finding a new theoretical route for incorporating the refractive index to have a correction factor. As a result, the accuracy of the orientation angle calculated by pMAIRS spectra has been significantly improved. In addition, with the improved technique, perfectly un-oriented molecules are readily discriminated from the oriented molecules with the orientation angle of 54.7, which has long been expected in material science.
Nano-Electrochemistry
Electrodeposition with Nano-Bipolar Electrodes in 2D and 3D Geometries

Bipolar electrochemistry has been used in examination of electrochemical effects under high electric fields, optically-coupled experiments, and geometries where conventionally-connected electrodes would be prohibitively complex. In macro-scale bipolar electrochemistry, the difference in electric potential between the electrode and the surrounding electrolyte provides the driving force for a chemical reaction. This research extends on previous work by examining the kinetics and underlying mechanistic behavior of nanoscale bipolar electrodeposition, which behave differently due to inherent limitations of nanoscale mass transport. A silver conductive filament is created by electrodeposition in a nanoscale gap between two electrodes, creating an atomic-scale junction. Junction behavior is characterized electrically in 2-dimensional geometries made using standard photolithography techniques and focused-ion beam (FIB), and in 3-dimensional geometries using conductive AFM. Junction morphology is examined using SEM. Preliminary results have demonstrated repeatable formation and dissolution of silver filaments in both geometries. With application areas ranging from massively-parallel chemical sensing arrays to non-contact tunable metamaterials, increasing the repeatability and mechanistic understanding of nanoelectrodeposition is important to advancing the use of similar technology in real-world devices.

Special thanks to the Defense Advanced Research Projects Agency (DARPA) and the NASA Space Technology Research Fellowship (NSTRF) for providing funding for this research.

Keywords: Atomic Force Microscopy (AFM), Electrochemistry, Nanotechnology, Sensors
Application Code: Nanotechnology
Methodology Code: Electrochemistry
Nano-Electrochemistry

Ion Selectivity Induced by Redox Cycling Within Nanopore Electrode Arrays at Weakly Supported Solution

Molecular transport in nanopore had attracted more and more attention due to its capability for efficient separation and ultra-sensitive analysis in chemical measurement areas. Electrochemical reactions happened inside nanopore electrode possess unique characteristics, e.g. fast mass transport, high signal-to-noise ratio, and insignificant ohmic losses, which inspired us to fabricate highly ordered nanopore arrays with precisely controlled pore size and vertically stacked metal-insulator-metal geometry for redox cycling reaction. These nanopore electrode arrays (NEAs) exhibited current amplification as large as 55-fold, collection efficiency over 99%, linear response ranging from 100 nM to 10 mM and small charging current with scan rate up to 100 V/s. Furthermore, these NEAs demonstrated a better understanding of ion transport near charged surface and high electric field at low ionic strength. By decreasing the electrolyte concentration, the thickness of electric double layer (EDL) was comparable to the inter-electrode distance. In the absence of electrolyte, we found both ion accumulation and ion migration contributed to ~200 times amplified current signal than the solution with fully supported electrolyte. The dependence of geometric parameters of NEAs (pore size or thickness of passivation layer) and the charge of analyte (charge number or polarity) on the redox cycling reaction of NEAs were also investigated. Finally, a ~3000 fold selectivity of dopamine (positively charged) versus ascorbic acid (negatively charged) was achieved by redox cycling-enabled ion accumulation or depletion within NEAs.

Abstract Text

Keywords: Electrochemistry, Integrated Sensor Systems, Microelectrode, Voltammetry
Application Code: Nanotechnology
Methodology Code: Electrochemistry
The study and detailed understanding of transient electrochemical processes can have positive impacts in both fundamental electrochemistry and highly sensitive electrochemical sensing. One of the most effective methods to study transient single nanoparticle (NP) collision events is the method of electrocatalytic amplification (EA). Based on the EA method, we have described the first observation of short current spikes during single NP collision in hydrazine solution. A current spike is defined as a sharp ~0.5 ms current pulse observed immediately after NP collision. The current quickly decays back to the steady-state diffusion limited hydrazine oxidation current on Pt NPs. The observation of such current spikes is facilitated by several key factors including a high NP concentration, and the use of light irradiation.

We have proposed a plausible mechanism for such current spikes. Nanobubbles of hydrogen are generated from catalytic decomposition of hydrazine on Pt NPs. These nanobubbles are attached to single Pt NPs and are quickly oxidized when a contact is made between the NP and the carbon UME. The presence of such nanobubbles is established and stabilized by continuous decomposition of hydrazine molecules on Pt and dissolution of H₂ from the nanobubble into the bulk solution. Our results show that these nanobubbles are about 25-nm in radii based on the total number of hydrogen molecules detected at the collision events. TEM imaging in a liquid cell revealed the presence of such gaseous structures confirming our hypothesis about hydrogen nanobubbles.

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Keywords: Analysis, Electrochemistry, Microelectrode
Application Code: General Interest
Methodology Code: Electrochemistry
Here we report the preparation, characterization, and electrochemical study of conductive, ultrathin films of cross-linked metal nanoparticles (NPs). Nanoporous films ranging from 40 to 200 nm in thickness composed of gold and platinum NPs of 5 nm were fabricated via a powerful layer-by-layer spin coating process. This process allows preparation of uniform NP films as large as 2 × 2 cm² with precise control over thickness, structure, and electrochemical and electrocatalytic properties. Gold, platinum, and bimetallic NP films were fabricated and characterized using cyclic voltammetry, scanning electron microscopy, and conductance measurements. Their electrocatalytic activity towards oxygen reduction reaction was investigated. Our results show that the electrochemical activity of such NP films is initially hindered by the presence of dense thiolate cross-linking ligands. Both electrochemical cycling and oxygen plasma cleaning are effective means in restoring their electrochemical activity. Gold NP films have higher electric conductivity than platinum possibly due to more uniform film structure and closer particle-particle distance. The electrochemical and electrocatalytic performance of platinum NP films can be greatly enhanced by the incorporation of gold NPs. This work focuses on electrochemical characterization of cross-linked NP films and demonstrates several unique properties. These include quick and easy preparation, ultrathin and uniform film thickness, tunable structure and composition, and transferable to many other substrates.

The authors gratefully acknowledge financial support from the National Institute of Health (GM101133) and University of Washington. Part of this work was conducted at the Molecular Analysis Facility which is supported in part by the National Science Foundation (grant ECC-1542101), the University of Washington, the Molecular Engineering & Sciences Institute, the Clean Energy Institute, and the National Institutes of Health.

Keywords: Electrochemistry, Electrode Surfaces, Nanotechnology
Application Code: Nanotechnology
Methodology Code: Electrochemistry
We demonstrate the first fabrication of closed bipolar nanoelectrodes (CBNE) with Pt nanoparticle as conductor electrode and quartz pipet as sheath/cell via direct FIB milling and drawing. The dimension and shape of the platinum nanoelectrodes can be easily controlled by the initial patterning process. With perchloric acid inside the quartz pipette, bipolar coupling can be achieved between proton reduction versus ferrocene oxidation in acetonitrile solution or ferrocene methanol oxidation in aqueous solution. The CV curves exhibit well-defined sigmoidal shape and the steady states currents also meet the theoretical prediction. Dual-nanoelectrodes can also be fabricated by using theta pipette. Electrochemiluminescence and electrochemical fluorogenic experiments were successfully carried out on Pt CBNEs. A 2x2 CBNE array were further fabricated and used for fluorescence-enabled electrochemical microscopy application. This versatile method for design and fabrication various bipolar electrodes and electrode arrays at nanoscale would be greatly beneficial to nanoscale electrochemical imaging area.

Keywords: Electrochemistry, Electrodes, Fluorescence, Nanotechnology

Application Code: General Interest

Methodology Code: Electrochemistry
Adsorbate identity and local surface features combine to control chemical transformations at electrocatalytic surfaces. Recently, we have expanded the in-situ toolbox to study their impact at photo- and electro-catalytic reactions on heterogeneous surfaces. Using nanoresolved in situ redox titrations, we characterize how surface modifications, such as defects or co-catalysts, locally impact adsorbate reactivity. In the Surface Interrogation mode of scanning electrochemical microscopy, after a catalytic step is performed to populate the surface with catalytic intermediates, we titrate the surface by electrochemically generating a titrant at the tip from a mediator in solution. Our first demonstration of spatially resolved redox titrations quantified adsorbed reactive oxygen species (ROS) formed on SrTiO$_3$ during photoassisted water oxidation. Using these measurements, we showed how substrate-adsorbate interactions at defective sites can dramatically decrease local ROS kinetics without influencing surface coverage. We also demonstrated the titration of 30 attomol of reactive oxygen species on the surface. Recently, we have developed strategies using novel redox mediators to image intermediates formed on Pt alloy oxygen reduction catalysts to show how dealloying and platinum dissolution create new adsorbate populations. Our studies, have pushed the capabilities of SI-SECM by performing titrations of fewer than a million molecules with nanoscale spatial resolution.


Keywords: Electrochemistry, Electrode Surfaces, Microelectrode, Semiconductor
Application Code: Material Science
Methodology Code: Electrochemistry
Hydrogen evolution reaction (HER), a cathodic half reaction of electrolytic water splitting, has been studied extensively for renewable electrochemical energy conversion and storage systems. Platinum, the most widely used HER catalyst, requires very low overpotentials with generating high cathodic current densities, but has the limitations of high cost and scarcity of supply. Various materials have been developed and characterized as electrocatalysts for HER.

In this work, we synthesize iridium-iridium oxide nanofibers (Ir-IrO\(_2\)NFs) by simple electrospinning of an iridium precursor/polymer solution and then calcination. The calcination is carried out at different temperatures ranged from 300 to 900 [degree]C. The morphologies, compositions and structures of Ir-IrO\(_2\)NFs calcined at various temperature are characterized by field emission scanning electron microscopy (FE-SEM), transmission electron microscopy (TEM) and X-ray diffraction (XRD). As the calcination temperature increases, the composition ratio of crystallized IrO\(_2\) in Ir-IrO\(_2\)NFs is increased and that of Ir metal is decreased. The electrochemical activities of Ir-IrO\(_2\)NFs are investigated by linear sweep voltammetry (LSV) using rotating disk electrode (RDE) measurement. The HER onset potential, Tafel slope and durability of synthesized Ir-IrO\(_2\)NFs are compared with the ones of platinum. For better understanding of the experimental results, HER catalyzed by Ir-IrO\(_2\)NFs is studied using density functional theory (DFT) calculations based on d-band theory.

This research was supported by the National Research Foundation of Korea (NRF) grant funded by the Ministry of Education, Science and Technology (NRF-2014R1A2A2A05003769).

Keywords: Atomic Absorption, Electrochemistry, Electrode Surfaces, Nanotechnology
Application Code: General Interest
Methodology Code: Electrochemistry
The commercial milling of wheat is accomplished by sequential unit processes involving 50 or more intermediate processing streams. Efficient separation of wheat endosperm from individual kernels to yield flour is achieved by close attention to detail by the professional miller in charge of the overall manufacturing operation. The efficiency of each individual separation unit process results in a cumulative effect that results in an optimum yield of product that meets the required purity specification. The analytical technique of quantitative chemical imaging allows the definition of the purity of millstreams as wheat endosperm in comparison to age old indirect methods. Multiplying the percent endosperm by the flow rate of respective individual streams provides the flow of endosperm for each unit process. The summation of these results is used to express the contribution of endosperm at various percentages to the cumulative yield for the overall process.

**Keywords:** Imaging, Process Monitoring, Quantitative, Spectroscopy

**Application Code:** Process Analytical Chemistry

**Methodology Code:** Vibrational Spectroscopy
Abstract Text

Usually the identification of components in mixtures is performed by wet chemistry, various chromatographic and optical spectroscopic techniques, which are effective yet time consuming, costly and require considerable scientific know-how.

To enhance this challenging process we developed a virtual method to rapidly analyze complex mixtures consisting of unknown substances based on a state-of-the-art concept of algorithms in combination with a comprehensive reference spectra database.

The presented application combines the virtual deconvolution of composites with automated matching and interpretation of their unknown components. It was especially considered that not only the existing search algorithms had to be improved and refined but also that the number of reference spectra has to be increased as a high matching quality significantly depends on the number of reference spectra.

Additionally the presented virtual methodology of analyzing mixtures gains in significance due to the rapid technological development of MIR & Raman spectrometers in terms of size and ease of use. This development is also accompanied by an increase of spectral data which has to be processed and interpreted with expertise in order to be of value.

The new and sophisticated combination of algorithms and the particularly large number of digital reference spectra results in a highly reliable performance of identifying the unknown components of mixtures even for unskilled users.

This innovative application is effective, cost saving and includes the required know-how to automatically identify the components and their quantities.

Keywords: Database, Identification, Infrared and Raman, Molecular Spectroscopy

Application Code: Other

Methodology Code: Molecular Spectroscopy
Ethylene oxide produced from ethylene epoxidation on Ag-based heterogeneous catalyst constitutes one of the largest volume chemicals in chemical industry [1]. Although the mechanism of this reaction has been investigated over decades, a detailed understanding of the reactant/catalyst interfacial chemistry is still highly desirable [2]. Surface enhanced Raman spectroscopy (SERS) is a powerful vibrational spectroscopy technique that enables the localized detection of low concentration chemical species with high sensitivity under in situ and ambient conditions [3]. Using SERS, we are able to track the adsorption behavior of ethylene molecules onto Ag nanoparticles and the formation of reactive intermediates/products on the scale of individual emitters. It is interesting to find that individual nanoparticles behave very differently in terms of adsorbed intermediates and products formed in the ethylene epoxidation reaction. Further characterization of this nanoparticle-to-nanoparticle heterogeneity in catalytic responses will help the understanding of ethylene/Ag interfacial chemistry and lead to chemical principles for improvement of catalytic selectivity of nanostructured Ag toward the formation of ethylene oxide.

Reference

Keywords: Material Science, Surface Analysis, Surface Enhanced Raman Spectroscopy, Vibrational Spectroscopy
Application Code: Material Science
Methodology Code: Vibrational Spectroscopy
Surface-enhanced Raman spectroscopy has a great potential as an analytical technique for on-site identification of chemical species, ranging from food additives in a complex food matrix to residual pesticides on agricultural produce. A powerful laser combined with a compact spectrometer constitutes a system, and requisite SERS surfaces are now readily available. What would propel the use of SERS forward is SERS surfaces tailored for specific applications. In this presentation, we describe our attempts for minimizing preprocessing of samples, i.e. separation of target molecules from a complex medium and removal of molecules from a solid surface. For the first application, we incorporated a SERS layer into a thin layer chromatography plate which would allow in-situ SERS characterization of a developed sample. More specifically, we show that melamine added to skim milk can be separated and its characteristic Raman peaks can be obtained. For detection of surface-adsorbed molecules, we prepared a flexible PDMS sheet onto which silver nanostructures are formed. Pressing the PDMS sheet onto a surface coated with target molecules could allow their transfer, allowing SERS characterization. A specific example is detection fungicides on the surface of a grape fruit.

While we typically use surface adsorbed SiO2 nanospheres coated with silver as the silver nanostructure, we have also developed additional methods for silver nanostructure fabrication based on the galvanic displacement reaction with AgNO3 solution as well as coating of naturally-existing nanostructures such as butterfly scales. It is hoped that a SERS surface can be optimized by selecting one particular fabrication method.

Keywords: Food Contaminants, Nanotechnology, Separation Sciences, Surface Enhanced Raman Spectroscopy
Application Code: Food Contaminants
Methodology Code: Vibrational Spectroscopy
Isotope labeled compounds and associated experiments play a key role in the investigation of absorption, distribution, metabolism and excretion (ADME) properties of new chemical entities as well as metabolic flux or quantitative bioanalytical analysis. The labeled compounds typically contain some amount of native compounds, the relative amount of which can introduce systematic errors in subsequent analysis and reported results. When measured with MS, there are severe MS signal overlapping between the labeled and un-labeled species, posing a quantitative challenge even on high resolution MS. In this paper, we propose a direct MS analysis approach to this problem through mass spectral accuracy analysis.

Two commercially available compounds, one with stable 2H labeling and one with multiple (uniform) 14C labeling, are used. Pure calibration standards are acquired under normal operating conditions on quadrupole LC/MS and under varying resolving powers on the LTQ/Orbitrap for MS calibration for spectral accuracy. Synthetic mixtures of the labeled and unlabeled compounds are acquired and calibrated with this MS calibration. With the spectral accuracy achieved through this calibration, the mutually overlapping MS signals from various labelled and un-labelled ion species in a mixture can be mathematically resolved through least squares regression, with accurate quantitative results for the relative concentration of each ion.

On quadrupole LC/MS systems, high mass spectral accuracy of >99.0% has been achieved with relative concentration determined down to 1.0% relative level and in great agreement (1.0% relative error) with the volumetric calculation. With the higher resolution Orbitrap data, the overall quantitative results are inferior to the quadrupole results, even when operating under optimal resolving power where the spectral accuracy of pure standards is the highest (>98.0%). Factors affecting the high resolution MS and possible remedies will be discussed.

Keywords: Isotope Ratio MS, Liquid Chromatography/Mass Spectroscopy, Organic Mass Spectrometry, Pharmaceutical
Application Code: Pharmaceutical
Methodology Code: Liquid Chromatography/Mass Spectrometry
In pharmaceutical and biopharmaceutical laboratories, the analysis of impurities is of great importance during the development of products. A common practice to identify unknown impurities involves manual fraction collection, followed by lengthy evaporative steps and multiple chromatographic steps. Multi-dimensional liquid chromatography (LC) can offer a solution to automate this process by diverting the peak of interest for additional characterization. One technique, known as “heart-cutting” is ideally suited for method development for impurity analysis of pharmaceuticals and biopharmaceuticals. “Heart-cutting” refers to the process where selected volumes of the first dimension effluent are diverted to a second column. When used with synchronized valve switching and tandem column configurations - such as ion exchange (IEX) / reverse phase (RP) - this technique can be used to heart-cut peaks of interest from the first dimension column to the second dimension column thereby taking advantage of different separation modes. This targeted approach enables versatility in impurities identification: it can be used for discovery of possible coeluting compounds by separation in the second dimension or to characterize the analyte by mass spectrometry (MS) for those analyses using non MS mobile phases. The later involves trapping the analyte of interest and replacing the mobile phase with a MS-friendly eluent at the second-dimension column. Samples of pharmaceutical interest will be used to demonstrate 2D-LC application in impurity analysis, and measures of chromatography performance will be presented.
A stability-indicating UPLC-UV method was developed for simultaneously identification and assay of Imidacloprid, Fipronil, s-Methoprene and BHT and estimation of Imidacloprid, Fipronil and s-Methoprene related compounds in a topical spot-on finished product. By the automated method development software – ChromSword®, one HPLC method was first developed, further fine-tuned and converted into an UPLC method. The final UPLC method is carried out using a BEH C18 column (2.1 mm I.D x 100 mm length, 1.7 µm particle size) maintained at 40 °C with mobile phase A (0.05% v/v of Phosphoric acid in water) and mobile phase B (acetonitrile/methanol, 60/40 v/v). Analytes are separated by a gradient elution, identified and quantitated by an external calibration against s-Methoprene and BHT standard solution at 280 nm. s-Methoprene and BHT are identified by matching their retention times in sample solution chromatogram to those in standard solution chromatogram. Imidacloprid, Fipronil and related compounds are identified by match their relative retention times vs. s-Methoprene to those listed in the method, respectively. Individual peaks for APIs and related compounds are quantitated by the external standard method against the s-Methoprene peak in the working standard solution. BHT is quantitated against the BHT peak in the working standard solution. The UPLC method was preliminarily validated per ICH guidelines and the stability indicating power of the method was evaluated by stressing the samples under acid, base, heat, light and oxidation. This method was used to support multiple probe stability studies for formulation development and clinical studies. Based on the knowledge of the authors through literature search, this is the first stability-indicating UPLC method for determination of Imidacloprid, Fipronil, s-Methoprene.
Delmopinol is an agent used in many human oral hygiene products such as mouth rinse and toothpaste over past several decades to prevent bacterial attachment to the teeth, gums, and tongue. Herein we report development of a fast Reversed-Phase High Performance Liquid Chromatography (RP-HPLC) method for assay of delmopinol in commercial batches of this product. A pH stable C18 HPLC column from Phenomenex (Gemini-nx, 50 mm × 4.6 mm I.D., 3 μm particle size) maintained at 35 °C was used in the method. Delmopinol samples were analyzed with an isocratic elution using 42% ACN/ 58% 5mM NH4OH as the mobile phase and the detection wavelength of 210 nm. The overall HPLC analysis time of this method is 8 minutes with the analyte eluting at around 5 minutes. The Limit of Quantitation (LOQ) of the method was established to be 0.05% of delmopinol target concentration. The method has been demonstrated to be robust for the slight change of HPLC parameters and is suitable for routine analysis of bulk delmopinol in a QC lab.
The aminoglycosides are a large and diverse class of antibiotics that are used in clinical and veterinary medicine and in agriculture. Their analysis is very important in the several application areas ranging from the impurities in antibiotics, pharmacokinetic and drug stability studies, development of pharmaceutical formulations, analysis of antibiotics residues in food and drug dissolution studies. The electrochemical detection in the combination with high-performance liquid chromatography is one of the most popular methods used for analysis of aminoglycosides.

In this presentation, we report our recent efforts in the optimization of methods based on high performance liquid chromatography with electrochemical detection for analysis of aminoglycosides. Several important method operating parameters influencing the performance of electrochemical detection have been investigated and optimized. We will compare the analytical performance optimized methods to those of existing EP and USP methods. We will demonstrate better linear calibration, enhanced signal-to-noise, and improved method stability for determination of various aminoglycosides obtained using the optimized methods.

Keywords: Bioanalytical, Electrochemistry, Liquid Chromatography, Pharmaceutical
Application Code: Pharmaceutical
Methodology Code: Liquid Chromatography
# Development of a RP-UPLC Method for Determination of Assay and Related Compounds of Betamethasone Valerate and Clotrimazole in a Topical Veterinary Drug Formulation

Betamethasone Valerate is an established anti-inflammatory agent. Clotrimazole is widely used as an antifungal agent. A combination of these two ingredients is used in a novel Topical Veterinary Drug Formulation intended for the treatment of fungal infections and inflammatory conditions in Pets. A reversed phase ultra-high performance liquid chromatography (RP-UPLC) method has been developed to determine the assay and related compounds of both active ingredients in this novel topical formulation.

Samples are dissolved in a diluent consisting of ACN/Water 80/20 with 0.025% Acetic Acid. Analytes are eluted vial gradient elution on a Waters BEH C18 column (100 mm x 2.1 mm I.D. 1.7 μm particle size) maintained at 40 °C on a Waters UHPLC system using a mobile phase system of 10 mM Potassium Phosphate Buffer pH 7.0 as mobile phase A and acetonitrile as mobile phase B at a flow rate of 0.5 mL/min. Both active ingredients and their known related compounds are fully separated in 10 minutes and Detected by UV at 239 nm. The quantitation is achieved by a single external reference standard calibration with a quantitation limit at 0.3%.

**Abstract Text**

Betamethasone Valerate is an established anti-inflammatory agent. Clotrimazole is widely used as an antifungal agent. A combination of these two ingredients is used in a novel Topical Veterinary Drug Formulation intended for the treatment of fungal infections and inflammatory conditions in Pets. A reversed phase ultra-high performance liquid chromatography (RP-UPLC) method has been developed to determine the assay and related compounds of both active ingredients in this novel topical formulation. Samples are dissolved in a diluent consisting of ACN/Water 80/20 with 0.025% Acetic Acid. Analytes are eluted vial gradient elution on a Waters BEH C18 column (100 mm x 2.1 mm I.D. 1.7 μm particle size) maintained at 40 °C on a Waters UHPLC system using a mobile phase system of 10 mM Potassium Phosphate Buffer pH 7.0 as mobile phase A and acetonitrile as mobile phase B at a flow rate of 0.5 mL/min. Both active ingredients and their known related compounds are fully separated in 10 minutes and Detected by UV at 239 nm. The quantitation is achieved by a single external reference standard calibration with a quantitation limit at 0.3%.

**Keywords:** Chromatography, HPLC, Method Development, Pharmaceutical

**Application Code:** Pharmaceutical

**Methodology Code:** Liquid Chromatography
Pharmaceutical Analysis by Liquid Chromatography

Development of a Stability Indicating RP-HPLC Method for Firocoxib Oral Suspension Solution

Firocoxib oral suspension solution is developed for managing pain and inflammation associated with osteoarthritis in horses and dogs. Firocoxib oral suspension contains Firoxib as an Active Pharmaceutical Ingredient (API) and Sodium Benzoate as a preservative. A reversed phase high performance liquid chromatography (RP-HPLC) stability indicating method has been developed for Firocoxib oral suspension solution. Oral suspension sample is extracted using ACN/H2O (80/20, v/v). The analytes are separated in 12 minutes by a gradient elution on an ACE 5 C18 column (50 mm × 4.6 mm I.D., 5µm particle size) maintained at 35 °C with mobile phase A of 0.1% phosphoric acid and mobile phase B of Acetonitrile. The analytes are detected with UV detection at 240 nm and quantitated against an external reference standard with a quantitation limit of 0.2% of target Firocoxib concentration in the oral suspension sample.

Abstract Text

Firocoxib oral suspension solution is developed for managing pain and inflammation associated with osteoarthritis in horses and dogs. Firocoxib oral suspension contains Firoxib as an Active Pharmaceutical Ingredient (API) and Sodium Benzoate as a preservative. A reversed phase high performance liquid chromatography (RP-HPLC) stability indicating method has been developed for Firocoxib oral suspension solution. Oral suspension sample is extracted using ACN/H2O (80/20, v/v). The analytes are separated in 12 minutes by a gradient elution on an ACE 5 C18 column (50 mm × 4.6 mm I.D., 5µm particle size) maintained at 35 °C with mobile phase A of 0.1% phosphoric acid and mobile phase B of Acetonitrile. The analytes are detected with UV detection at 240 nm and quantitated against an external reference standard with a quantitation limit of 0.2% of target Firocoxib concentration in the oral suspension sample.

Keywords: Extraction, HPLC, Pharmaceutical, Separation Sciences
Application Code: Pharmaceutical
Methodology Code: Liquid Chromatography
Detection of uranium is critical for the identification of trafficked materials, prevention of nuclear proliferation, monitoring of environmental contamination, and for determining drinking water quality. The best current practices for analysis of trace amounts of uranium are inductively coupled plasma mass spectrometry (ICPMS) and thermal ionization mass spectrometry (TIMS). These techniques, however, are not ideal for field analysis because of a lack of portability, a high cost of maintenance of the instruments, and the requirement of a highly trained analyst for the operation of the instrument. In this work we demonstrate a time insensitive assay for the detection of trace amounts of aqueous uranium based on surface-enhanced Raman scattering. This is accomplished by the use of glutathione functionalized silver colloids to selectively bind uranyl ions in solution. Binding is indicated by the presence of a vibrational mode unique to the uranyl ion in the SERS spectrum at 834 cm$^{-1}$ which can then be used for quantitative analysis. This method yields a linear range from approximately 50-200 ppb and delivers a limit of detection of 24 ppb. We further demonstrate that the functionalized colloids remain active more than three months after preparation and quantify uranium in the presence of interferences likely to be present in groundwater. To our knowledge this is the first study using a portable hand-held Raman instrument for analysis of uranium. Additionally, our limit of detection of 24 ppb surpasses the United States Environmental Protection Agency acceptable drinking water concentration of 30 ppb in drinking water.

**Keywords:** Environmental Analysis, Molecular Spectroscopy, Nuclear Analytical Applications, Surface Enhanced

**Application Code:** Environmental

**Methodology Code:** Vibrational Spectroscopy
Surface enhanced Raman spectroscopy (SERS) is a powerful technique capable of probing single molecules with unprecedented detail for processes ranging from biological sensing and chemical transformation to optical waveguides and electronics. Unfortunately, most currently used SERS substrates contain <10 nm gaps between gold or silver particles, in which probe molecules are inserted. Although this strategy often leads to single molecule detection of the probe molecule, it also results in denaturation and inactivation of these molecules, which severely limits the use of SERS for biophysical measurements. In the Sagle group, we are working with novel liposome-based SERS substrates, in which probe molecules are encapsulated in the liposome component, rather than tethered to or sandwiched between metallic surfaces. Initial characterization of these substrates shows high field enhancement of 107-108, and good measurement reproducibility. Measurements of substrate fidelity and temperature changes associated with laser illumination are also carried out and show the substrates are robust. Lastly, the biocompatibility of these substrates is evaluated through incorporation of horse heart cytochrome c, and comparison of the structure and function to that of the same protein in solution.

Keywords: Bioanalytical, Infrared and Raman, Nanotechnology, Surface Enhanced Raman Spectroscopy

Application Code: Bioanalytical

Methodology Code: Vibrational Spectroscopy
Common practice in the application of surface-enhanced Raman spectroscopy (SERS) examines aggregated spheroidal nanoparticles of noble metals as the enhancing substrate, enabling the amplification of the Raman fingerprint from the target molecule. This approach, which generally provides good results with fair reproducibility, suffers from lack of optimization between laser wavelength and nanoparticle extinction maximum, reducing the applicability of SERS.

The aim of this research is to exploit the knowledge of the parameters regulating the extinction function of metals, such as chemical identity, size, and shape, to synthetically and post-synthetically implement a colloid-based substrate with optimized extinction maximum and surface environment to be used in the forensic toxicological field.

As substantiated by theory, shape has been confirmed to be a crucial aspect when tuning the extinction maximum for lasers in the red and NIR regions, and rapid one-pot synthetic pathways for anisotropic morphologies have been explored, optimized, and scaled up as means of maximizing the technique-required pre-resonance conditions between the enhancing substrate and Raman source.

This synthesis-based methodology for the implementation of a SERS protocol constitutes a novel approach in forensic toxicology, and appears promising in the perspective of promoting SERS as a new, robust analytical-toxicological tool, potentially providing a cheaper alternative to current strategies, such as LC-MS and immunoassays.

Major support for this research has been provided by award #2015-IJ-CX-K006 from the NIJ. Points of view in the document are those of the authors and do not necessarily represent the official view of the US department of Justice.
Aggregation of proteins containing expanded polyglutamine tracts is associated with at least 10 neurodegenerative diseases, including Huntington’s disease. Given that monomeric conformational transitions are thought to play a role in the initial stages of aggregation and neurotoxicity, it is important to understand the solution-state conformation(s) of polyglutamine peptides. We investigated the solution-state structure of a model polyglutamine peptide, \(D[2Q10K2] (Q10)\), using UV resonance Raman (UVRR) spectroscopy coupled with metadynamics simulations. Reexamination of the UVRR spectrum of non-disaggregated \(Q10\) (NDQ10) shows that it forms a collapsed beta-strand in solution, rather than a beta-hairpin as previously thought. In contrast, disaggregated \(Q10\) (DQ10) forms PPII and 2.5\(\alpha\)-helix structures in aqueous solution. We use the secondary structure-inducing cosolvent acetonitrile to investigate additional \(Q10\) conformational propensities. We find that \(Q10\) adopts a soluble \(\alpha\)-helical structure in solvents containing 50%-80% acetonitrile and aggregates in solvents containing >80% acetonitrile. We compare our experimentally derived solution-state structures of \(Q10\) to that observed in metadynamics simulations, used to investigate the conformational landscape of \(Q10\). The simulations show energy wells containing PPII/2.5\(\alpha\)-helix, \(\alpha\)-helix, and collapsed beta-strand structures consistent with experimental results. We quantitatively compare the Ramachandran [\(\Psi\)] angle distributions obtained from UVRR to that observed in the metadynamics simulations and find excellent agreement. To our knowledge this is the first computational study of solution-state polyglutamine directly validated by experimental results.

Keywords: Biospectroscopy, Molecular Spectroscopy, Raman Spectroscopy, Vibrational Spectroscopy
Application Code: Bioanalytical
Methodology Code: Vibrational Spectroscopy
We developed a novel wide-field deep UV Raman hyperspectral imaging spectrometer for standoff explosive detection. Our imaging spectrometer utilizes a crystalline colloidal array photonic crystal to select narrow spectral regions of deep UV Raman light for imaging and detection. The photonic crystal is composed of ~35 nm diameter monodisperse, highly charged silica nanospheres that self-assemble in solution to produce a face centered cubic crystal that diffracts deep UV (<300 nm) light governed by Bragg’s Law. We utilize a defocused 229 nm laser beam generated by a frequency doubled Argon ion laser to excite 2.3 m distant explosive samples and collect the Raman scattered light with a deep UV optimized Cassegrain telescope. After collimating the collected Raman light, we angle tune the photonic crystal to diffract and select deep UV Raman spectral regions containing Raman bands from explosives of interest. We focus the diffracted light onto a thermoelectrically cooled CCD detector to collect a wide-field Raman image that details the location of explosive species within the region illuminated by the laser. We have demonstrated detection and imaging of 10 g/cm^2 inkjet printed samples provided by the Army Research Lab of solid ammonium nitrate (NH\_4NO\_3) and pentaerythritol tetranitrate (PETN) on aluminum test substrates.

**Keywords:** Detection, Forensics, Imaging, Raman Spectroscopy

**Application Code:** Homeland Security/Forensics

**Methodology Code:** Molecular Spectroscopy
SERS is particularly effective in examining mono-molecular layers coating metal surfaces. The type of metal and the nano-structural geometries of planar SERS substrates will influence not only the binding affinities of the analytes but also plasmonic enhancement. To probe these influences, we are studying the formation of mono-molecular layers for many nitrogen and sulfur containing aromatic chemicals on planar SERS substrates of varying geometries and noble metal layers. Our studies will reveal the combined influence of metals and geometries on the effectiveness of planar SERS substrates as a tool for trace detection.
Nanoporous Silver Film Fabricated by Oxygen Plasma: A Facile Approach for SERS Substrates

Surfaced-enhanced Raman spectroscopy (SERS) demonstrates remarkable sensitivity for chemical and biochemical analysis, providing vibrational information for identification and quantitative determination of various analytes. Substrates play a key role in SERS-based sensing schemes and many different approaches to substrate creation have been explored. Substrates with the highest enhancement factor often require expensive and time-consuming lithographic approaches. Nanoporous metal films represent effective SERS substrates owing to their homogeneity, large surface area, and abundant hot-spots. Herein, we present a facile procedure for fabricating nanoporous Ag film on various substrate surfaces. Thermally deposited Ag film was treated with oxygen plasma, resulting in porous silver oxide film with nanoscale feature, which can be reduced to nanoporous Ag film (AgNF) with similar morphology. The AgNF substrate demonstrates 30 folds higher Raman intensity than Ag film over nanospheres using 4-mercaptobenzoic acid as the probe molecule, yielding an enhancement factor of \(6 \times 10^6\). The AgNF substrate was evaluated for benzoic acid, 4-nitrophenol, and 2-mercaptoethanesulfonate, showing high SERS sensitivity for chemicals that bind weakly to Ag surface and molecules with small Raman cross-section at µM concentration. In addition to its simplicity, the procedure could be applied to various supported materials such as transparency film, filter paper, polystyrene film, and aluminum foil, revealing similar Raman sensitivity. These results demonstrate the advantage of the proposed approach for mass production of low-cost, sensitive SERS substrates. The transferable nature of these AgNF to different flexible surfaces also allows their easy integration with other sensing schemes.

Keywords: Nanotechnology, Raman Spectroscopy, Surface Enhanced Raman Spectroscopy, Trace Analysis

Application Code: Other
Methodology Code: Vibrational Spectroscopy
Blood is a very complex mixture housing many bio-macromolecules including metabolites (According to Human Serum Metabolome Database (www.hmdb.ca) more than four thousand metabolites) released from cells. In principle, an abnormality in a group of cells can be identified from their metabolites circulating in blood, which are mainly proteins as the end products of gene expression. However, their very low concentration makes it very difficult for easy detection. The other point is competitive adsorption of molecules on nanoparticle surfaces based on their molecular structure. Analyte concentration, nanoparticle concentration and pH directly influence spectral outcomes in multiplex SERS studies. In this regard, concentration of metabolites in blood and used nanoparticle concentration as SERS substrate give final shape of blood SERS spectrum. In this study, we have systematically investigated source of the bands observed on blood SERS spectrum with the aim of finding clues for cancer diagnosis. First, proteins of blood serum (collected from healthy individuals and cancer patients) are separated and remaining aqueous parts are analysed using SERS to investigate any difference for cancer diagnosis. Then, protein parts are analysed. The results indicate that the SERS can provide at least complementary information to increase reliability of cancer diagnosis.
The human breath contains volatile and non-volatile compounds of clinical interest. Also, breath sampling is attractive because it is non-invasive and unlimitedly repeatable, as opposed to blood, urine and tissue. Thus, analysis of exhaled human breath has great potential for rapid clinical diagnosis and drug monitoring.

Plasma-based ambient ionization/desorption MS, emerging as a frontier technology, is characterized by soft ionization, fast analysis, and little-to-no pretreatment requirement. Also, it has the advantage of more efficient ionization of low molecular weight compounds over a relatively wide polarity range. In addition, it may be combined with any MS instrument with an ambient inlet to perform on-site breath analysis in clinics.

Previously, an LTP-MS method was successfully developed for real-time, in-vivo quantitative analysis of valprolactone, a metabolite of valproic acid (antiepileptic), in exhaled breath without any sample pretreatment. The concentration of valprolactone in exhaled breath was determined for the first time to be 4.83 (± 0.32) ng/L. Nevertheless, accessibility of mass spectrometers may still limit the wide applicability of the real-time analysis approach.

Here, with the aim of overcoming the above mentioned limitation, the application of LTP-MS for offline quantitative analysis of exhaled breath will be presented. Exhaled breath is collected on a filter which is then exposed to the LTP effluent for desorption/ionization. The working conditions (including geometry of LTP source, filter paper material, etc.) will be optimized to obtain the best analytical figures of merit for different analytes of interest in exhaled breath (valprolactone, eucalyptol, nicotine and propofol, etc.).

Keywords: Clinical/Toxicology, Mass Spectrometry, Pharmaceutical, Plasma
Application Code: Clinical/Toxicology
Methodology Code: Mass Spectrometry
Abstract Text
Here we apply picosecond infrared laser (PIRL) ablation to bulk water under atmospheric conditions for the soft extraction of small molecule, peptides, proteins and protein complex ions for direct mass spectrometry (MS) analysis. Molecular extraction is performed under desorption by impulsive vibrational excitation (DIVE) conditions, utilizing a picosecond infrared laser pulse tuned to excite the O-H vibrational stretching mode of water, to drive analyte extraction and desolvation faster than the thermal and acoustic relaxation times of the excited volume. The PIRL-DIVE excited volume is fully thermally and acoustically confined minimizing energy transfer and has previously been utilized for near scar free surgery as well as demonstrated to efficiently extract structurally and functionally conserved biomolecules and complexes directly from tissue and solution. Here we utilize PIRL-DIVE ablation to produce highly charged biomolecules in the gas phase for high-sensitivity MS detection. Notably, the method is capable of producing both negatively and positively charged ions, without the use of a secondary ionization device, over a broad pH range with species detected at low amol concentrations. Near-physiological sample conditions were utilized to characterize the soft nature of impulsive PIRL-DIVE extraction with proteins in their native state as well as noncovalently bound protein ligand complexes produced directly from water. The results are particularly applicable to top down proteomics. Further, PIRL-DIVE is capable of operating at low sample consumption rates (~30 picoliters per laser pulse) and its flexibility demonstrated by the fast sampling of a nanofabricated aqueous sample sorting device.

Keywords: Lab-on-a-Chip/Microfluidics, Mass Spectrometry, Method Development, Protein
Application Code: Biomedical
Methodology Code: Mass Spectrometry
The field of illicit drug testing has recently become a constantly changing environment with the rapid development of unregulated designer and synthetic compounds. These compounds are reported to generate stimulating affects similar to that of methamphetamine, heroin and MDMA. The difficulty for forensic testing facilities is the fact that these compounds are not detected under normal ELISA testing methods; therefore, more specific LC-MS based approaches are necessary. This study demonstrates the benefits of Biocompatible Solid Phase Micro Extraction (BioSPME) used in conjunction with direct mass spectrometry detection. An ambient ionization source (DART-Direct Analysis Real-Time) was coupled to a single MS system (Waters QDA). This technique offers a fast, accurate, and robust method for analyzing drugs of abuse. Linear calibration curves were observed for all analytes in full scan mode from 100-5000 ng/mL. Limits of detection were between 15-20 ng/mL and quantitation limits were between 50-100 ng/mL. The urine matrix did not negatively impact the analyte responses from the fiber. Using biocompatible SPME fibers with DART-MS on the Waters QDA provides customers a more cost effective analysis alternative compared to the more expensive LC/MS/MS systems.
### Abstract Text

The crystallin proteins are a major component in the lens of the eye; a primary function of alpha-crystallin is to prevent denaturation and aggregation of proteins in the lens through chaperoning functions. When crystallin function is lost cataracts can form in the eye due to the aggregation of the proteins which are present in the lens. UV light exposure during a person's lifetime can cause a loss of chaperoning ability in crystallin proteins. This study provides evidence on the effect that UV light has on the overall chaperoning ability of alpha-crystallin. Ficoll is used as a crowding agent in the study to determine if molecular crowding can protect the crystallin from losing function. The results of the aggregation assays for chaperoning function as well as protein structure insight from fluorescence spectroscopy will be presented.

### Keywords
- Biomedical
- Fluorescence
- Protein
- Spectroscopy

### Application Code
- Biomedical

### Methodology Code
- UV/VIS
Impact of Polyphenolic Compounds on the Structure and Aggregation of the Amyloid-β Peptide

Aggregation of the amyloid-β peptide is associated with the development of Alzheimer’s disease. Aβ is a 39-43 residue cleavage product of the amyloid precursor protein (APP). Aβ aggregates to produce insoluble plaques in the brain, which are composed of cross-β sheet structured fibrils. Various polyphenolic compounds have been shown to interfere with Aβ aggregation. The interaction of Aβ with the naturally occurring polyphenolic compounds nordihydroguaiaretic acid, curcumin, resveratrol, and piceatannol were investigated via circular dichroism (CD), and deep-ultraviolet resonance Raman (dUVRR) spectroscopies. Polyphenols have comparable structural characteristics, such as length, aromatic rings, and hydroxyl group substituents. Altering these structural components could play a part in their ability to inhibit Aβ aggregation. CD and dUVRR were employed to determine if inhibitors and non-inhibitors had similar effects on Aβ structure. Interactions with the aromatic residues of the peptide were observed.

Keywords: Biospectroscopy, Fluorescence, Protein, Raman Spectroscopy
Application Code: Bioanalytical
Methodology Code: Biospectroscopy
It is known that membrane-embedded α-helices are more uniform structurally than their aqueous counterparts. Despite this uniformity, distortions and localized unfolding are thought to be common in these proteins in order for them to conduct their cellular tasks. However, how amino acid sequence facilitates these conformational shifts remains unknown, as methods for investigating structural heterogeneity in transmembrane proteins are limited. Circular dichroism (CD) is often used to characterize the secondary structure of proteins, but the broadly overlapped spectral features limit its usefulness. Deep-ultraviolet resonance Raman spectroscopy (dUVRR) is an emerging structurally sensitive spectroscopic technique for analyzing membrane protein structure. The backbone amide modes are resonance enhanced in dUVRR spectra while the lipid features are not resulting in strong membrane protein spectral features in near native environments.

Using model leucine-alanine peptides in increasingly dehydrated (aqueous, surfactant and bilayer) environments, hydration dependent changes in the amide modes were characterized. The amide I mode in the dUVRR spectra of these peptides increased with increasing dehydration, while the amide III decreased. These results indicate that the dehydration of the peptide backbone is accompanied by and increase in helical structure. Incorporation of helix breaking residues (HBR), proline or glycine, in these model peptides promoted helical instability in lipophilic environments.

Keywords: Amino Acids, Membrane, Peptides, Raman Spectroscopy
Application Code: Bioanalytical
Methodology Code: Biospectroscopy
Recently, DNA based keypad lock systems provide a new approach for protecting information at the molecular scale. While the complexity of design and the mismatch among DNA would make the molecular logic more complicated for designing, which is more prominent in constructing DNA keyboard lock. Regarding the property of narrow peak width and the ability of multi-channel output, Surface Enhanced Raman Scattering (SERS) has shown great potential to enhance the output-reading techniques. We have developed a new DNA keypad lock based on the host and guest AuNPs system, in which SERS was used as a readout tool for the first time. The multichannel output reading method contributes to designing a more functional system. Two kinds of Raman dyes were introduced to represent the final outputs. One is cresol violet (CV), which helps to transmit the “unlock” signal. The other is malachite green (MG) which is used to transmit the “locked” signal. Subsequently, two kinds of correspond host GNPs were design. One of them named O1 helps to confirm the “open permissions” signal. The other named O2 is labelled by MG, which carries the “locked” signal. The strategy that reading the output results in a variety of channels avoids the uncertainty influence of the more DNA strands, and makes it ideal for building a more complex DNA keypad lock with more inputs. This strategy can be used in building a more functional keypad lock based on the different spectral fingerprints of Raman dyes which is called permission lock.
Surface-enhanced Raman scattering (SERS) is an emerging analytical technique for the detection and identification of chemicals and biological molecules and structures with sensitivity down to single molecule. Rapid, sensitive and accurate identification of bacteria is critical not only clinical diagnostics but also industrial applications. Several studies have been demonstrated that SERS can be used as powerful technique for the detection and identification of bacteria using various sample preparation methods and SERS substrates. Sample preparation and SERS substrates are critical factors to obtain strong, sensitive, and reproducible SERS spectra for detection and identification of bacteria. In this study, we report label-free detection and identification of bacteria on plasmonic silver nanodomes (AgNDs) structures. AgNDs are fabricated by combining of soft lithography and nanosphere lithography. First, convective-assembly method is used for the uniform deposition of the latex particles (1600 nm) on a glass slide. After the PDMS is poured on the latex thin film to obtain nanovoids on the PDMS surface. The prepared nanovoids are used as template for the fabrication of AgNDs. Finally, the nanovoids are filled with Ag by electrochemical deposition to obtain AgNDs. E. coli, P. aeruginosa, S. aureus, and B. pasteurii are used as model bacteria to test the performance of the AgNDs. The results indicate that AgNDs not only improve the spectral quality but also increase the reproducibility of the SERS spectra.

Keywords: Surface Enhanced Raman Spectroscopy
Application Code: Bioanalytical
Methodology Code: Vibrational Spectroscopy
Among the variables that are appropriate for direct feedback control of the perfusion rate in mammalian cell cultures, high priority should be given to the glucose concentration. Here we describe the application of a closed-loop control scheme for the long-term cultivation of CHO cells in a high cell density (35 – 40 million cells/ml) perfusion process. The monitoring and control system worked successfully for 2.5 months without any signs of performance degradation. In targeting industrial applications, issues such as reliability, sterility and accuracy are given high priority. The implementation of the glucose monitoring system, which is the main part of the control complex, is addressed. The performance of the perfusion culture was evaluated at four different glucose set points, providing essential information about process optimization. The online glucose concentration was used by an embedded expert system which drove the process through the batch and the perfusion phase, achieving total SCADA control of the feed rate. In summary, the proposed glucose monitoring and control technique proved to be a reliable tool which can be applied with confidence at an industrial scale for either microbial or mammalian cell cultures.
Bioanalytical - Others

Surface-Enhanced Raman Spectroscopy Detection of Biomolecules Using AgNPs Attached Filter Paper Substrates Array

In biomedicine and bioanalytical chemistry, a simple, low cost and rapid analytical method is of critical importance for identifying various clinical microorganisms in biological samples. In this work, we demonstrate a surface-enhanced Raman scattering (SERS) detection method for biomolecules detection using filter paper SERS substrates array. The proposed SERS method is essentially a label-free vibrational technique associated with silver nanoparticles and its SERS enhancement toward biomolecules detection. A simple chemical method, silver mirror reaction, has been used to prepare silver nanoparticles (AgNPs) on filter paper substrates. To obtain huge SERS enhancement, filter paper substrates were pretreated with ammonium hydroxide to attach large number of silver nanoparticles on the filter paper surface. The morphology of our SERS substrates was characterized by scanning electron microscopy (SEM) images. Based on the SEM images, a large number of AgNPs are uniformly distributed on the surface of filter paper substrates. First, silver nanoparticles were attached on filter paper substrates using silver mirror reaction. Second, AgNPs attached filter substrates were used to form a SERS substrates array on a glass slide. Finally, a certain volume of target sample was deposited on the substrates array for SERS detection. The as-prepared SERS substrate array allows mass analysis of aqueous samples with a requirement of only 5 μL in sample volume. Importantly, the as-prepared SERS substrates exhibit significant SERS enhancement in the detection of various probe molecules such as non-structural protein (NS1), mycoplasma pneumoniae and ureaplasma. This kind of low cost SERS substrates array has great potential as a portable sensor for on-site biological or chemical analysis.

Keywords: Bioanalytical, Medical, Nanotechnology, Surface Enhanced Raman Spectroscopy
Application Code: Bioanalytical
Methodology Code: Vibrational Spectroscopy
Gangliosides are glycosylated sphingolipids that reside primarily in the extracellular leaflet of plasma membranes. In most tissues gangliosides are present at low levels (~1% of lipids), but in the nervous system gangliosides are present at levels approaching 10% of total lipids. The major gangliosides found in the brain and CNS are GM1, GD1a, GD1b, and GT1b. These molecules share the same general head group structure, but are differentiated by the number and position of pendent sialic acid groups. At physiological pH, sialic acid carries a negative charge, which imparts a charge on gangliosides that varies from (-1, GM1) to (-3, GT1b). Gangliosides function as receptors for toxins and viruses, as well as a number of endogenous biomolecules. As such, supported lipid bilayer (SLB)-based biosensors have been used to detect molecular interactions with gangliosides. However, the influence gangliosides have on the adsorption and rupture of phospholipid vesicles to form SLBs has not been characterized in detail. In this work we used quartz crystal microbalance with dissipation monitoring (QCM-D) to measure the kinetics of SLB formation from vesicles containing varying levels of GM1, GD1a, GD1b, and GT1b. We found that all the gangliosides tend to slow SLB formation kinetics in a concentration and charge dependent manner, and the critical surface vesicle concentration increases with increasing ganglioside concentration and charge. Additionally, calcium can accelerate the formation of SLBs with gangliosides, and the nature of the SLB hydration layer may be altered by the presence of gangliosides.

Keywords: Bioanalytical, Biosensors, Lipids, Surface Analysis
Application Code: Bioanalytical
Methodology Code: Surface Analysis/Imaging
Bioanalytical - Others

Discrimination of Human and Animal Blood Traces Via Raman Spectroscopy

The species identification of bloodstains is an important and immediate challenge for forensic science, veterinary purposes, and wildlife preservation. In particular, determining the origin of a bloodstain is a critical, yet overlooked, step in establishing its relevance to the crime. The current methods used to identify the species of origin of bloodstains are limited in scope and destructive to the sample. We have previously demonstrated that Raman spectroscopy can reliably differentiate blood traces from three species: human, cat, and dog. The research presented here demonstrates that multivariate statistical analysis of near infrared Raman spectroscopic data can be effectively applied as a nondestructive technique for differentiating human blood from a wide survey of animal blood, in a binary fashion, and as a means to classify the individual species. The developed approach does not require the knowledge of a specific (bio)chemical marker for each species class but rather relies on spectroscopic statistical differentiation of various components. Several performance measures, including a blind test and external validation, confirmed the discriminatory performance of the chemometric models developed. This approach resulted in remarkable classification ability even with intrinsically heterogeneous classes and samples. These findings further demonstrate great potential for using Raman spectroscopy in the field of serology, especially for species identification of suspected bloodstains.

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Abstract Text

Keywords: Biological Samples, Chemometrics, Forensics, Raman Spectroscopy

Application Code: Bioanalytical

Methodology Code: Chemometrics
Investigation of Fluidity and Phase Segregation of Polymerized Mixed Planar Supported Lipid Bilayers for Biosensor Applications

Fluidity and stability are key characteristics of planar supported lipid bilayers (PSLBs) used as platforms for protein-based biosensors. PSLBs 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 membrane fluidity. To enhance bilayer stability while maintaining fluidity, mixed PSLBs composed of mixtures of bis-SorbPC (polymerizable lipid) and DPhPC (fluid lipid) were prepared and analyzed. Lateral diffusion coefficients (D) was measured as a function of bis-SorbPC/DPhPC molar ratio using fluorescence recovery after photobleaching (FRAP). D of PSLBs with 70% poly(bis-SorbPC) was observed to be in the same order of magnitude as of pure DPhPC PSLBs. This retention of fluidity even when the poly(bis-SorbPC) fraction is substantial, suggests that these bilayers are phase segregated, and composed of polymerized and fluid domains. Domains were not observed with fluorescence microscopy, thus atomic force microscopy (AFM) was employed to investigate the sub-µm morphology. Nano-scale phase segregation of the two lipids was observed. DPhPC forms a continuous lipid matrix surrounding the island-like poly(bis-SorbPC) domains. With AFM force spectroscopy, breakthrough force for DPhPC domains was observed to be 5 nN, whereas no breakthrough was observed for poly(bis-SorbPC) domains, implying that protein insertion into polymer domains might not be possible. In summary, polymerized mixed PSLBs form nano-structured membranes which are fluid and stable thus showing considerable potential as platforms for protein-based biosensors.

Acknowledgements: NIH R01EB007047, NSF 1337371

Keywords: Atomic Force Microscopy (AFM), Biosensors, Lipids
Application Code: Bioanalytical
Methodology Code: Surface Analysis/Imaging
Carnosine is an endogenous dipeptide (consisting of alanine and histidine) that has been shown to have antioxidant properties by scavenging reactive nitrogen and oxygen species in the body (RNOS). RNOS are important stress mediators and lead to the activation of macrophages via the immune response. Therefore, the interaction between macrophages cells and carnosine is of great interest. A microchip electrophoresis with laser induced fluorescence detection (ME-LIF) method was developed in order to separate carnosine from other prominent intracellular amines. Carnosine was derivatized with naphthalene-2,3-dicarboxaldehyde and had limits of detection of 65.5 nM in standards. Using this method, carnosine was detected in basal macrophages. Additionally, the cellular uptake of carnosine was investigated under pro-inflammatory conditions via stressing the cells with lipopolysaccharides and interferon-γ. There was a 2.8-fold increase in carnosine concentration in the stressed macrophages, showing that macrophages in pro-inflammatory environments will uptake antioxidant molecules as a defense mechanism. Under these conditions, RNOS will also cause the conversion of arginine to citrulline. The ME-LIF method was modified to allow for these amines to also be detected so that their ratio could be investigated in basal and stressed cells incubated with carnosine and see if it influences the conversion ratio.
Microfluidic paper-based analytical devices (PADs) are very useful for chemical and biochemical detection of several analytes. 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. PADs offer many advantages such as: aqueous solution transport by capillarity, porosity to store reagents, light weight for easy handling, straightforward fabrication of microfluidic channels and low cost. All these features make PADs ideal for disposable, onetime use tests. Raman scattering originates from inelastic scattering of light from molecules that can be correlated to the vibrational modes, producing molecular fingerprints. Surface enhanced Raman scattering (SERS) provides enhancement of the Raman scattering from molecules that interact with SERS-active nanostructures. The combination of SERS capabilities with PADs provides for higher levels of signal amplification for detection and quantification of several analytes. In our group we are developing a PAD with SERS capabilities. High surface area Ag nanostructured dendrites are made by electrodeposition into the paper and provide enhancement of the Raman signal. The combination of SERS-PADs and commercial portable Raman spectrometer will provide inexpensive chemically selective detection of analytes in mixtures.

Keywords: Bioanalytical, Electrochemistry, Nanotechnology, Raman Spectroscopy
Application Code: Bioanalytical
Methodology Code: Microfluidics/Lab-on-a-Chip
Bioanalytical - Others

Graphene Oxide-Based biosensor for Rapid and Sensitive Detection of HIV-1 Protease

In this work, we report a GO-Pep-fluorescein fluorescence resonance energy transfer (FRET) biosensor for the rapid, sensitive and accurate detection of HIV-1 protease, in which fluorescent labeled substrate peptide of HIV-1 protease was covalently linked to GO. In the absence of HIV-1 protease, fluorescein is effectively quenched by GO. However, in the presence of HIV-1 protease, the substrate peptide will be cleaved, thus producing fluorescence. Based on this sensing mechanism, the HIV-1 protease can be detected as low as nanogram per milliliter. More importantly, the sensor could accurately detect HIV-1 protease in human serum. Such GO-based FRET sensors may have useful applications in many fields, including diagnosis of protease-related diseases, as well as sensitive and high-throughput screening of drug candidates.

Keywords: Biosensors, Fluorescence, Protein, Sensors

Application Code: Bioanalytical

Methodology Code: Fluorescence/Luminescence
### Abstract Title

**Albumin Removal from Human Serum Using Selective Nanopockets on Silica-Coated Magnetic Nanoparticles**

Selecting removal of albumin from human serum is an essential step prior to proteomic analyses, especially when using mass spectrometry. Here we report stable synthetic nanopockets on magnetic nanoparticle surfaces that bind to human serum albumin (HSA) with high affinity and specificity. The nanopockets are created by templating HSA on 200 nm silica-coated paramagnetic nanoparticles using mixed polymer layers of 4 organo-silane monomers. These monomers have amino acid-like side chains providing hydrophobic, hydrophilic and H-bonding interactions that closely mimic features of binding sites of an antibody. The binding capacity of the material was 20 mg HSA/g, and consistently removed ~80% albumin from human serum over multiple uses.

**Keywords:** Materials Characterization, Molecular Spectroscopy, Protein, Spectroscopy

**Application Code:** Bioanalytical

**Methodology Code:** Surface Analysis/Imaging
Ligand-receptor interactions drive all biological processes. Understanding ligand-receptor binding chemistry is important to modulate receptor-mediated signaling pathways as well as to develop receptor-targeted therapeutics. Tip-enhanced Raman spectroscopy (TERS) has emerged in recent years as a powerful tool to provide chemical insights of interacting biomolecules, by generating structural specific Raman spectra of single molecules. We have previously demonstrated that two integrin receptors with similar structures can be differentiated in intact cell membrane, due to slight differences in their ligand binding sites. Here we show that integrin β3 binding with three small peptide ligands, which share the same binding site on the receptor, give rise to different TERS spectra. The spectral differences indicate that not only the chemical nature of ligand-receptor binding pocket but also the binding conformation would affect the detected TERS signal. These conformational changes would also affect the binding affinities and kinetics that can be detected with surface plasmon resonance (SPR) and single particle tracking (SPT) experiments.

Keywords: Bioanalytical, Biospectroscopy, Raman Spectroscopy, Surface Enhanced Raman Spectroscopy
Application Code: Bioanalytical
Methodology Code: Biospectroscopy
Study of the microbiome is largely based on the description of thousands of genomic sequences generated from ensembles of microbes that are taken from complex populations. Inevitably the sequences generated from abundant organisms dominate the data. For example, in a population consisting of two species, one present at 1% of the other; roughly 300 cell equivalents would need to be sequenced in order to likely detect and identify the rarer species. More than 99% of the sequences would be identical and from the dominant species. This conventional approach is inefficient and expensive. More crucially, the inference of unique genome sequences from the mixed data are impeded due to the similarity of related genera resulting in large ambiguity about the exact depth of the microbiome.

Separating the microbial population into reduced-complexity fractions prior to deep-sequencing is the most straightforward approach to improving the microbiome. Conventional approaches use flow-cytometry to bin the microbiome. This approach reduces the complexity of each fraction but does not enrich a fraction; hence the depth of the microbiome is limited and highly abundant organisms contribute to all fractions. By contrast, electrophoretic fractionation separates microbes by chemical properties resulting in enrichment of rare microbes.

We have developed an instrument that interfaces capillary electrophoresis with a sterile fraction collector to address this unmet need of preparative separations of microbiome populations. Microbial populations, and extended studies on bacteriophage will be separated and deposited into wells of microtiter plates for sequence analysis.

Keywords: Capillary Electrophoresis, Environmental/Soils, Isolation/Purification, Sample Handling/Automation
Application Code: Bioanalytical
Methodology Code: Capillary Electrophoresis
Ultrasound-Assisted Microemulsion Electrokinetic Chromatography has been investigated as a new capillary electrophoresis technique. UAMEC specifically assists the separation of neutral species or species with similar electrophoretic mobilities that cannot be separated using conventional capillary zone electrophoresis (CZE). In this type of electrokinetic chromatography, a pseudostationary phase consisting of an immiscible organic solvent is dispersed by ultrasonic waves in the aqueous running buffer. Partitioning then occurs between the very tiny organic solvent droplets and the aqueous buffer phase. The analytes which have higher solubility in organic solvent will spend more time dissolved in organic phase and exhibit higher migration times, while those with higher solubility in the aqueous phase will elute faster and have shorter migration times. Preliminary results demonstrated that L-DOPA-tryptophan, catechol-aminophenol, and dopamine-serotonin pairs could not be resolved with conventional CZE, but were resolved successfully with the UAMEC technique. Resolution for L-DOPA-tryptophan, catechol-aminophenol, and dopamine-serotonin pairs were determined to be 5.45, 1.74, and 1.61, respectively. 1,2 dichloroethane was selected as the pseudostationary phase based on low solubility in water, high dispersibility, stability of the dispersed solvent, and low volatility. The pH of running buffer was adjusted to 8.0 using 40 mM phosphate buffer solution based on preliminary optimization.

Keywords: Bioanalytical, Capillary Electrophoresis, Detection, Electrophoresis
Application Code: Bioanalytical
Methodology Code: Capillary Electrophoresis
We have developed a method for tissue analysis using matrix-assisted laser desorption ionization (MALDI) mass spectrometry imaging for region of interest selection followed by parallel liquid chromatography tandem mass spectrometry (LC MS/MS) analyses for proteomics identification and quantification and DNA and RNA analysis with regards to expression quantification, sequencing and potential modifications detection. This multi-omics method combines the high throughput and spatial resolution of MALDI imaging with the quantification and identification capabilities of proteomics LC MS/MS and genomics sequencing. Rat brain tissue sections ranging from 10 to 50 µm in thickness are sectioned and mounted on a conductive microscope slide. The matrix is pneumatically sprayed and images are obtained with a commercial MALDI time-of-flight mass spectrometer. Regions of interest are identified using the heat-map mass image and infrared laser ablation sampling is used to transfer material to a capturing solvent. While the same section used for MALDI analysis can be sampled to obtain material for downstream analyses, consecutive sections can be used to obtain technical replicate or increase available material. Bottom-up proteomics nanoLC tandem mass spectrometry is performed on part of the captured sample using an ion trap mass spectrometer. The rest of the sample is amplified and sequenced to evaluate genetic composition. Preliminary data demonstrate that DNA as well as proteins RNA can be transferred without lo completely intact and with the chemical structure of each monomer preserved. Laser ablation transfer has been demonstrated both with frozen as well as with formalin fixed paraffin embedded tissue samples. Ongoing work is aimed at automation of the region selection as well as infrared laser ablation sampling and developing workflows for imaging mass spectrometry combined with detailed genomics and proteomics studies.
Engineered nanoparticles are increasingly incorporated in a wide variety of polymeric materials with favorable matrix-filler interactions that resulted in improved physical, chemical, and electrical properties. Environmental weathering and the potential release of carbon nanotubes (CNTs) from composite matrices becomes a concern as a high volume application of nanocomposite is coming real. It is essential to understand the relationship between the inherent characteristics of nanocomposite based consumer products and the likelihood of the release of nanomaterials throughout the life-cycle of the product. In the present study nanocomposite films and wafers of polyolefins, polyimide and epoxy were exposed to accelerated weathering conditions. The changes in the physical, chemical, and structural properties of these composites and their potential for the release of nanomaterials were investigated. Pristine and CNT-added polymer film were aged in a weathering chamber that simulated UV exposure, humidity and rain cycles. The role of chemical nature of polymer, film thickness, and exposure condition were correlated with the degradation and release of CNTs. The weathered samples were characterized by SEM, TEM, DSC, TGA, XRD, FTIR and optical microscopy techniques. The release of CNTs from polymer-CNT composite were measured using dynamic light scattering and single particle-ICP-MS. The results of the characterization and nanoparticle release as well as the toxicity of the release nanoparticles will be discussed in this presentation.
Characterization of Polymers and Plastics

Rapid Measurement of Molecular Weight by a Novel GPC Column

The column design is the most important point to achieve rapid measurements. The biggest hurdle to overcome is the high back pressure generated by high flow rates which constrains the column design. Polymer gel has been preferably used for size exclusion chromatography (SEC) separation because of its variety of pore sizes, but has not been packed in columns for rapid measurement due to the concern that the high back pressure might crush the polymer gel, not allowing the column to work. In order to solve the problem, the authors have developed a porous cross-linked styrene-divinylbenzene polymer gel with sharp particle size distribution to pack the Shodex HK column. The polymer gel successfully works to suppress back pressure, high flow rate, and GPC column for rapid measurement has been realized with the polymer gel. The GPC column can be used for molecular weight measurement with the range from 100 to 1 million Da. The column constructed with a 4.6 mm inner diameter and 150 mm length dimension, has elution volume with less than 2.0 mL. It corresponds to less than 2.0 minutes of measurement time in case of 1.0 mL/min flow rate. At this flow rate, back pressure reaches 13.8 MPa, a suitable level for conventional HPLC equipment. Polycarbonate as well as other polymers', molecular weight measurements were studied at various flow rates. The results obtained the same values at 1.0 mL/min and 2.0 mL/min flow rate. This shows SEC mode separation, can be achieved even under more rapid conditions.

Keywords: Biopharmaceutical, HPLC, HPLC Columns, Polymers & Plastics
Application Code: Polymers and Plastics
Methodology Code: Liquid Chromatography
Automotive coatings are multi-layer film structures typically consisting of an electrocoat, primer, base coat, and clear coat. Each layer of the paint system has different functions contributing to the overall performance and appearance of the coating system. In traditional coating process, each layer is sprayed individually and baked separately. More recently, wet-on-wet coating processes have gained popularity due to the significantly shortened flash and baking times, resulting in lower energy costs and greater productivity for auto manufacturers. One drawback to the wet-on-wet process is that the appearances of the coatings are often inferior to coatings applied using the traditional process. Migration of certain chemical species between the adjacent layers was believed to be one of the factors for the decreased appearance. With a spatial resolution as low as 2 µm, attenuated total internal reflectance (ATR) FTIR microspectroscopy was utilized to track possible chemical migration across mounted and cross-sectioned multilayer coating systems.
Characterization of Polymers and Plastics

New Techniques for Preparing Plastics and Polymers by Microwave Sample Preparation

Traditional acid digestion of many polymers have typically involved the use of a sulfuric acid char step in order to dehydrate the polymer and break the cross linkages. This is followed by a second step of oxidation with nitric acid. This is a tedious process and the sulfuric acid step raises the viscosity of the liquid which is not friendly to ICP-MS analysis. Other techniques such as dry ashing samples prior to taking up in dilute acids and very costly high pressure apparatus have also been attempted but each has their own difficulties.

We will prepare a variety of polymeric samples by traditional cost effective microwave digestion without the use of sulfuric acid or dry ashing steps. Analysis will be reported using ICP-OES and ICP-MS analysis. Spike recovery data as well as data derived from standard reference materials will presented and discussed.

Keywords: ICP-MS, Metals, Microwave, Petrochemical
Application Code: Polymers and Plastics
Methodology Code: Sampling and Sample Preparation
## Abstract

The global polypropylene (PP) market is the second largest volume polymer business in the world today making up 25% of global polymer demand. PP analysis is challenging because it requires high temperature for dissolution. Both GPC columns and GPC system with complex hardware design must be suitable for high temperature operation. A robust method of high temperature analysis showing consistency in sample preparation of high crystalline structure of polypropylene yielding molecular weight reproducibility is critical for the end user.

Here we report the determination of molar mass averages and polydispersity of 2-polypropylene random copolymer using two High Temperature GPC columns from Tosoh with exclusion limit $4 \times 10^8$ g/mol. Samples (2.5 mg/mL) were prepared by dissolving portions of the polymer in trichlorobenzene at 160°C for 4 hours. The EcoSEC High Temperature GPC System from Tosoh equipped with dual flow RI detector was used. The mobile phase and solvent were trichlorobenzene with butylated hydroxyltoluene (BHT).

Preliminary results show that the calibration curve was fitted with a cubic function with <5% error. The chromatogram of each of the samples was monitored by dual flow refractive index detector yielded superb signal to noise ratio as well as an extremely stable RI baseline. No significant variation was observed in the sample retention times between injections. The number, weight, and z-average molar mass values ($M_n$, $M_w$, and $M_z$), and polydispersity index, PDI, were calculated for both of PS and PP equivalents using EcoSEC software with applying Mark-Houwink constants. The analysis yielded exceptional reproducibility.

**Keywords:** Chromatography, HPLC Columns, Liquid Chromatography, Polymers & Plastics

**Application Code:** Polymers and Plastics

**Methodology Code:** Liquid Chromatography
Ultra-high molecular weight (MW) polymers are widely used as dewatering aids, flocculants, and rheology modifiers in various industries such as water treatment, paper making, mining, and energy services. Molecular weight is one of the most important properties influencing polymer performance in such applications. Because of the ultra-high MW of these polymers, their MW characterization is very challenging.

In this work, a batch-mode multi-angle light scattering (MALS) method was developed for the MW determination of ultra-high MW polymers. The principles of batch-mode MALS, method development considerations, and the results on the analysis of two important types of ultra-high MW polymers, dextran and poly (acrylamide) are presented. The results show that batch mode MALS can be successfully used to determine the MW for ultra-high MW polymers.
Mobile fuel storage containers use a flexible coated fabric composite to store military fuel for all Army tactical vehicles. These storage containers are composed of a high strength woven fabric that has been coated on both sides by a fuel resistant rubber coating. Due to the inherent nature of the coating, these structures lose fuel very rapidly through chemical diffusion of the individual fuel molecules into the coated fabric and subsequently into the atmosphere. In these experiments, it is possible to use time resolved FT-IR-ATR to follow the diffusion of fuel as it migrates through these polymer membranes. These data are then used to generate real time kinetic profiles which allow calculation the diffusion coefficients, $D$, for this process using the Barbari-Fieldson model. The model uses the integrated IR absorbance of the appearing individual fuel IR bands to estimate concentration of fuel at the crystal-polymer interface. This concentration gradient allows use of Fick’s second law to calculate $D$. 

Keywords: FTIR, Materials Characterization, Polymers & Plastics

Application Code: Polymers and Plastics

Methodology Code: Vibrational Spectroscopy
Most cosmetics and personal care products contain fragrance analytes that may be allergens or skin irritants to some consumers. To be compliant with various regulations, manufacturers are required to provide information on the presence of specific allergens when exceeding a specified concentration threshold. GC-TOFMS is well-suited for this type of targeted screening and also simultaneously provides non-targeted characterization for better general understanding of a product. A GC-TOFMS method was developed to complete a fast screening for 25 contact allergens in approximately 5 minutes by using a short (10m) and narrow (0.18mm) Rxi-17Sil MS column (Restek) and a fast temperature ramp. Chromatographic separation together with mathematical deconvolution of the TOFMS data reliably separated all of the target allergens. The method was used to develop calibration equations for all standards from 1 ppb to 1 ppm (on-column) with excellent linearity and correlation coefficients. A variety of commercially-available perfume and cologne samples were screened for the target allergens with the calibrations rapidly providing quantitative information. Because TOFMS provides full mass-range data, this approach simultaneously accomplishes non-targeted analyses, so additional characterization and comparison of the perfume samples were also performed.

Keywords: Calibration, Consumer Products, GC-MS, Time of Flight MS
Application Code: Consumer Products
Methodology Code: Gas Chromatography/Mass Spectrometry
Formaldehyde is a colorless strong-smelling gas used in tiny amounts as preservative in cosmetics and personal care products to prevent mould and bacteria, but is considered a potential carcinogenic agent at a certain concentrations.

In this work the static headspace extraction method coupled with GC/TOF-MS analysis is described to determine PFBHA derivatized formaldehyde in cosmetic products.

Coupling a static headspace with a new automatic sampler guarantees a good repeatability in terms of automatic STD addition and derivatizing agent ensuring a complete automation of all operation steps, and a strong reduction of sample handling.

Furthermore, the capability of a TOF-MS detector provides high quality mass spectra and an effective deconvolution algorithm ensures a highly reliable identification, even with a complex matrix as a cosmetic product.
Green synthesis is becoming increasingly popular in industry and research because it removes harsh organic solvents or can increase energy efficiency of reactions. This project will utilize green techniques and investigate new complexes with coumarin-3-carboxylic acid (CCAs) and lanthanide metals. There is a lack of knowledge on complexes of CCAs with europium(III) specifically. There is available data on the other luminescent and emission properties, but not on the phosphorescent values of CCA based-europium(III) complexes. The information that has been determined, such as the crystalline structures and characterization, will be used to fill in the lack of knowledge on the Eu(III)-CCA complexes and their phosphorescent properties.

Keywords: FTIR, Luminescence, NMR, X-ray Diffraction
Application Code: Consumer Products
Methodology Code: X-ray Techniques
**Session Title:** Food Safety  
**Abstract Title:** Accessible and Efficient Screening of Multiclass Contaminants in Food  
**Primary Author:** Kenneth J. Rosnack  
**Author:** Waters Corporation  
**Co-Author(s):** Eimear McCall, Jinchuan Yang, Joe Romano  
**Date:** Wednesday, March 08, 2017  
**Time:**  
**Room:** Exposition Floor, Aisle 2500-2600

### Abstract Text

With growing global trade and varying international regulations, the need for multi analyte screening procedures to efficiently detect violating residues is ever increasing. Routine testing laboratories continue to strive for efficient and reliable sample throughput methodologies, where generic analytical conditions are essential. SPE extracts of foodstuffs were run on UPLC separation using a BEH C18 (1.7 µm, 2.1x100 mm) analytical column. Targeted multi reaction monitoring methods were used to identify and quantify multiple compounds in a single method. This work reports the development of a screening method for the determination of multi-class multi-residue contaminants in complex foodstuffs, including foods of animal origin. A generic and simplified sample extraction protocol was used with liquid-liquid extraction, followed by a generic reverse phase, solid phase extraction (SPE) procedure. All solvent standards, matrix matched calibration curves and sample extracts were analyzed using UPLC coupled with a high sensitivity tandem quadrupole mass spectrometer.

The benefit of the high sensitivity instrument was evaluated, in order to investigate the effect of matrix dilution to overcome common analytical challenges such as ion suppression. Further enhancements in analytical efficiencies were achieved utilizing the functionality of the novel multi-mode ionization source, operated at atmospheric pressure. Exploiting the key functionality of the high velocity droplet stream extends the scope of multi-class analytes screened to excelled levels of detection in a single workflow.

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

**Application Code:** Food Safety

**Methodology Code:** Liquid Chromatography/Mass Spectrometry
One mission of fuel ethanol producers is to achieve zero discharge of process water into the environment. In order to accomplish this goal, plants typically recycle water streams back into various points of the process where the influence on final product quality is minimal. Boiler water has historically been directly recycled into the corn cook step since additives are typically dosed at minimal levels and the components are generally recognized as safe (GRAS) for their intended use in the boiler. The Food Safety Modernization Act (FSMA), however, has required evaluation of potential hazards in distiller’s dried grains (DDG) used as animal feed, including boiler water additives (BWA) deposited from recycling practices.

This paper will present the challenges and results for the analysis of boiler water additives in DDG. The development of the methods and their detection limits was guided by predictive boiler engineering models and the known toxicology of the additives. A variety of measurement techniques was used, including reversed-phase and size-exclusion chromatography, as well as UV, fluorescence, and mass spectrometric detection. Boiler blowdown, whole stillage, and DDG samples were then acquired from a multi-plant study to evaluate the fate of the additives. Based on the acquired data, the regulatory implications of these findings will be discussed.

Keywords: Chromatography, Fluorescence, Food Safety, Liquid Chromatography/Mass Spectroscopy
Application Code: Food Safety
Methodology Code: Liquid Chromatography
Alcohol occurs naturally as a product of fermentation from the juice/beverage due to some microorganism. It became an important indicator when the microorganism was not well-controlled during production or/and transportation. AIJN (European fruit juice association) set specific limitations of alcohol level for fruit juice as ingredient quality evaluation. And for halal certification, alcohol is strictly forbidden for food and beverage producing and processing.

Traditionally, alcohol content was tested by GC (Gas Chromatography), which is widely applied as a quantitative approach in industry. Different than that, a fast and practical screening method is important and have big needs for routine beverage quality monitoring.

A fast screening method on alcohol has been developed. Alcohol can be determined simultaneously with an analysis method used to determine sugar content in beverage. This method are mainly applied for quantifying sugars such as sucrose, glucose, fructose, sorbitol and mannitol by liquid chromatography/refract meter (LC-RID) detection. Meanwhile, detection of alcohol in beverage with this method can also applied as a fast screening approach for alcohol monitoring. Report Limit of alcohol is 100mg/L. This method can realize multi-component determination including alcohol fast screening. Testing efficiency is improved especially for beverage quality control. As for sample preparation, no complicated pre-treatment of sample is required. It is easily to be carried out for alcohol fast screening for beverage industry applying this non-complicated but efficient method.

Keywords: Beverage, Food Safety, HPLC, HPLC Detection
Application Code: Food Safety
Methodology Code: Liquid Chromatography
With the globalization of our food supply, concerns about food quality and safety have led to an increased need for fast and sensitive analytical techniques. A wide array of inorganic ions, organic acids and carbohydrates can be analyzed by ion chromatography. For optimal results, high quality reagents are required, including the water used to prepare eluents, blanks, and dilute standards and samples. This water should be free of all the analytes tested, as they may interfere with analytical results, and should also be free of large organics, particles and bacteria since these water contaminants may interfere with the instrument itself (columns, suppressors...). In this study we investigated the suitability of a combination of water purification technologies in delivering water for ion chromatography analyses of ions commonly measured to assess food safety, quality, flavor, or to follow regulations. As a proof of principle, sodium, potassium, calcium (important dietary components), nitrate and nitrite (commonly analyzed in drinking water and processed meats), sulfate, fluoride, chloride, and organic acids such as acetate and lactate (indicators of spoilage of fruit juices) were analyzed using an ICS-3000 system with an eluent generator. Twelve different anions could be separated in a single run, and calibration curves were obtained in the 0.1-1 to 0.4-4 µg/L range, with RSD values <7% and R^{2} values >0.99. In conclusion, water purified with a combination of filtration, reverse osmosis, electrodeionization, ion-exchange, activated carbon and photo-oxidation is optimally suited for ion chromatography analyses.
Ultraviolet (UV-C) irradiation is a non-thermal disinfection method that is an alternative to heat pasteurization of beverages. This study investigated the effect of UV-C irradiation on the polyphenolic content of green tea. Catechins are a class of polyphenols and are the major constituents of green tea. UV-C irradiation doses ranging from 0 to 240 mJ•cm$^2$ were delivered to green tea, and the catechins were quantified. An LC-MS/MS method was developed to determine the concentrations of the following catechins: (+)-catechin (C), (-)-epicatechin gallate (EC), (-)-epigallocatechin (EGC), (-)-epigallocatechin gallate (EGCG), (-)-gallocatechin (GC), and (-)-gallocatechin gallate (GCG) in green tea. The LC-MS/MS method used a 2.6 micron superficially porous particle C18 column and a gradient elution program to separate the catechins with a 3.5 minute cycle time. The results indicated that UV-C treatment of green tea at relevant disinfection doses did not cause significant degradation of catechins in the green tea. Overall, these results demonstrate the effectiveness of the UV-C technology for treating highly turbid liquids such as green tea.
It is well known that speciation analysis is a mandatory tool in order to correctly address the potential risks associated with for example As in fish or food or Cr in drinking water. Due to the ionic nature of the most commonly investigated species, ion chromatography hyphenated to inductively coupled plasma mass spectrometry (IC-ICP-MS) is normally the method of choice. The striking advantage of this combination is that modern IC systems are completely metal free, so that background contamination caused through the chromatographic system can be systematically avoided. However, also liquid chromatography can help to unravel the distribution of a given element over different chemical compounds, normally using reversed phase separations for non-ionic compounds with different polarities. A typical example for the combination of HPLC to ICP-MS is the analysis of Sulfur and Phosphorous containing peptides or proteins. This presentation should highlight the advantages and applications of those chromatographic techniques when used in combination with ICP-MS as a powerful detection system for trace elements.
Analysis of pesticide residues in food is typically time-consuming due to the separation for multiple pesticides with a wide range of polarity and matrix co-eluting issues. To deal with the ever-growing number of pesticides, food safety laboratories need to ideally screen as many compounds as possible in a short time which may exceed maximum residual limits; typically 10 ppb in food matrices. In this study, we developed an analytical method for over four hundred pesticides in food matrices in SFC/MS.

A Nexera UC system coupled to triple quadrupole mass spectrometer (Shimadzu, Japan) was used. Separation was achieved using an ODS column embedded polar group with carbon dioxide and methanol containing 1 mM of ammonium formate as a modifier. Vegetable samples, with dry ice, were finely ground by milling until it became a powder and then extracted by a modified QuEChERS method. In SFC/MS, total flow rate of mobile phase is usually less than LC because supercritical CO2 is vaporized before MS, and water is not contained in the modifier. Therefore, ionization efficiency in ESI is generally increased. In this study, we observed that 90% of compounds showed better sensitivity in SFC/MS compared to common LC/MS analysis. 441 compounds with a wide range of polarity were successfully separated within 30 minutes and detected at 10ppb with enough sensitivity in SFC/MS. The matrix samples spiked with 10 ppb standards were prepared for the recovery. Most of target compounds have shown excellent recoveries ranging from 70 to 120% in the matrices.

Keywords: Food Safety, Pesticides, Quadrupole MS, Supercritical Fluid Chromatography
Application Code: Food Safety
Methodology Code: Supercritical Fluid Chromatography
Mycotoxins are low-molecular-weight natural products produced by fungi and are capable of causing disease and death. They are strictly regulated around the world because of their strong carcinogenic effects. A simple and reliable method to analyze mycotoxins is required to ensure food safety. The current methods require time-consuming sample pretreatment. Here we report a fully automated online sample extraction and analysis of mycotoxins in foods by online SFE-SFC-MS. Several mycotoxins such as Afla (aflatoxin B1, B2, G1 and G2), OTA (ochratoxin A), ZON (zearalenone), DON (deoxynivalenol) in foods were analyzed in this study. 1g of wet food samples were mixed with 1g of absorbent before loading to the extraction vessels, while 1g of dried food samples were put into the vessels as is. The vessels were set on the online SFE-SFC-MS system. The mycotoxins in food samples were successfully extracted from the samples by Nexera UC online SFE-SFC system, analyzed on the Cosmosil pi-NAP column, and detected by LCMS-8060.

Keywords: Food Safety, SFC, SFE
Application Code: Food Safety
Methodology Code: Supercritical Fluid Chromatography
UHPLC-MS applications are frequently developed on 2.1 mm I.D. columns at fairly high flow rates in the range of 0.3-1.0 mL/min. Lower flow rates promise better sensitivity and lower detection limits in mass spectrometry due to easier solvent removal and better ionization efficiency. Simply reducing the flow rate on the same column geometry would worsen the separation efficiency and reduce both chromatographic resolution and sensitivity. The use of 1.0 mm I.D. columns instead of 2.1 mm I.D. columns offers the advantage that the same LC performance can be generated at substantially lower flow rates, additionally reducing mobile phase consumption and hazardous waste production. On top, micro-flow UHPLC applications based on 1.0 mm I.D. columns will show a higher sensitivity compared to normal flow UHPLC, supposing the injected analyte mass stays the same.

When scaling down a method this theoretical increase of sensitivity follows the ratio of the squares of the internal column diameters. Thus, scaling a method down from 2.1 mm I.D. to 1.0 mm I.D. would lead to a sensitivity increase of $2.12/1.02 = 4.4$ for concentration-sensitive detectors, which mostly applies to mass spectrometry with electrospray ionization (ESI) sources. However, if UHPLC methods with 1.0 mm I.D. columns are coupled to MS detection, the sensitivity increase can deviate substantially from the theoretical value due to the specific physical chemical properties of the analytes influencing ionization efficiency, or mismatch of fluidic dimensions such as the emitter ID. Next to the MS, also the fluidic design and the instrument performance of the UHPLC system need to keep pace with the requirements claimed by the use of 1.0 mm ID columns.

In this study, we evaluate the compatibility of the latest state of the art in UHPLC instrumentation and Triple Quadrupole Mass Spectrometry with 1.0 mm I.D. columns for the trace-level analysis of pesticides at (sub-)ppb level.
Milk is especially vulnerable to aflatoxin contamination, as it can be easily ingested and concentrated during a cow’s grazing/feeding. When ingested by cows, aflatoxin B1 is converted to aflatoxin M1, which, though less potent than B1, has been shown to cause liver cancer in certain animals. [sup]1[/sup] As M1 is considered the primary aflatoxin expected to be found in milk, the European Union (EU) has established a stringent control limit for M1, set at 0.05 ppb in milk. [sup]2[/sup] This is currently the strictest global control limit in this regard, setting a significantly lower level than the Food and Drug Administration’s (FDA’s) limit of 0.5 ppb. [sup]1[/sup]

Addressing the above concern, we describe an HPLC-FL method for monitoring aflatoxin M1 in raw milk at ppb/ppt levels, using simple immunoaffinity solid phase extraction (SPE) methodology for initial sample preparation and clean-up. Though the focus herein was on aflatoxin M1, aflatoxins B1, B2, G1 and G2 were also included as part of the standards and spikes to confirm the chromatographic separation of M1 from other aflatoxins.

1) FDA Compliance Policy Guide, under Inspections, Compliance, Enforcement, and Criminal Investigations; CPG Section 527.400 Whole Milk, Lowfat Milk, Skim Milk - Aflatoxin M1.


Keywords: Food Safety, HPLC
Application Code: Food Safety
Methodology Code: Liquid Chromatography
Medicinal marijuana is a rapidly emerging industry in much of the United States of America. Laboratory analysis of the cannabis plant material, extracted resins and infused products is becoming a hot topic in this infant industry without national regulation.

The key goal for cannabis analysis is the safety of the consumer. Multiple analytical techniques are utilized to perform several different cannabis analyses to include potency, terpenes, residual solvents, heavy metals, pesticides and microbiological.

**Keywords:** Food Safety, GC, HPLC, ICP

**Application Code:** Food Safety

**Methodology Code:** Education/Teaching
Use of Liver Homogenates for Rapid Generation of Phase I Metabolites to Facilitate Characterization of Emerging Drugs of Abuse by High Resolution Liquid Chromatography-Mass Spectrometry

Microsomal or S9 fractions are typically used to produce drug metabolites for preliminary in vitro metabolism investigations and the development of targeted mass spectrometric methods. However, liver microsomes or S9 fractions are not readily available for many species and the production thereof is tedious, time-consuming and specialized equipment is needed. Simple, rapid and cost-effective in vitro strategies that can promptly respond to analytical challenges in the control and monitoring of emerging illegal drug use in the livestock sector are therefore required. The aim of this work was to investigate the potential of bovine liver homogenates to rapidly generate and characterise phase I metabolites of selective androgen receptor modulator (SARM) compounds. Bovine liver homogenate was generated by homogenization of fresh pooled liver tissue followed by low-speed centrifugation. In contrast, bovine liver microsomes were isolated from the same tissue samples through homogenization, followed by medium and high-speed centrifugation. After incubation of homogenate or microsomes (1 mg/mL) with a representative SARM compound (ostarine) and NADPH, metabolite compounds were concentrated by protein precipitation and liquid-liquid extraction. Analytes were then separated and identified by ultra high performance liquid chromatography (UHPLC) coupled to quadrupole time-of-flight mass spectrometry (QToF-MS). Parent compound (ostarine) and associated metabolites, including hydroxy-, bishydroxy-, O-dephenyl, O-dephenyl-demethyl ostarine, hydroxybenzonitrile and bishydroxybenzonitrile, were detected following incubations with either homogenate or microsomal preparations. The use of liver homogenate was demonstrated to be an effective alternative approach for use within in vitro drug metabolism based strategies facilitating the development of food safety focused targeted mass spectrometry detection methods.

Keywords: Bioanalytical, Drugs, Food Safety, Liquid Chromatography/Mass Spectroscopy
Application Code: Food Safety
Methodology Code: Liquid Chromatography/Mass Spectrometry
Investigation of the Primary Plasticizers Present in Polyvinyl Chloride (PVC) Products Currently Authorized as Food Contact Materials

PVC is a common food contact material that is usually plasticized to increase its flexibility. Phthalates are one of many chemical compounds that are often used as plasticizers in PVC but not necessarily in food contact materials. They may be used in packaging materials for foods and can also be found in components of certain food processing equipment such as conveyor belts and tubing. Transfer of phthalates from packaging to the surfaces of foods can occur. In recent years, there has been interest in understanding the health effects of phthalates, as well as the possible human exposure levels. However, there is limited information available about the routes of exposure to phthalates. In July 2014, the Chronic Hazard Advisory Panel (CHAP) produced a report for the U.S. Consumer Product Safety Commission (CPSC) detailing the hazards of phthalates and phthalate alternatives. This report listed diet as one factor contributing greater than or equal to 10% of total phthalate exposure. As a result of this report, the U.S. Food and Drug Administration (FDA) is interested in determining the types of the primary plasticizer present in food packaging and processing materials as well as their concentrations. An investigation was conducted of thirty six different samples of PVC food packaging and food processing materials using a solvent extraction and GC-MS analysis. Eight different plasticizers including two phthalates, DEHP and DIDP, were identified in the products tested. The plasticizer concentrations ranged from 1- 38% depending on the types of food contact materials and the type of plasticizer. The FDA has some information about the quantity of phthalates in food but will use this information to increase its understanding about distribution of plasticizer concentrations in food contact materials. Various types of plasticizer surveys have been conducted in other countries, such as the EU and Canada, but not in the US.

Keywords: Food Safety, Gas Chromatography/Mass Spectrometry, Polymers & Plastics
Application Code: Food Safety
Methodology Code: Gas Chromatography/Mass Spectrometry
### Abstract Text

The thyroid hormones 3,5,3’-triiodothyronine, commonly known as liothyronine (T3) and 3,5,3’,5’-tetraiodothyronine, commonly known as levothyroxine (T4) are produced in the thyroid gland where they are generally bound in the thyroglobulin protein. These iodine-containing hormones play an integral role in regulating cellular activity, growth and brain development. Our laboratory has previously analyzed dog treats and dietary supplements for the presence of T3 and T4, suspected to be originating from the inclusion of thyroid glands, using LC-MS/MS and LC-ICP-MS. These methods are capable of detecting lower levels present in these samples. However, protein-bound reference standards for the T3 and T4 hormones are not commercially available. This quality control material is necessary for validating current quantitation methods.

An internal quality control material (IQCM) was prepared from a thyroid prescription drug which contains known concentrations of both the T3 and T4 hormones bound in protein. The IQCM was analyzed multiple times using the current United States Pharmacopeia (USP) high performance liquid chromatography ultraviolet detection (HPLC-UV) method to determine T3 and T4 content. The IQCM was tested for homogeneity and moisture content. The now-characterized IQCM was used to ascertain the efficacy of existing digestion methods currently in use in our laboratory to release protein-bound T3 and T4. The IQCM was analyzed by HPLC-UV concurrently with the previously mentioned dog treat and dietary supplement samples. The resulting data led to an optimized digestion method for quantitation and facilitated a comparative study between samples containing high levels of T3 and T4.

### Keywords:
- Food Contaminants
- Food Safety
- Food Science
- Validation

### Application Code:
- Food Safety

### Methodology Code:
- Liquid Chromatography
Recent legislation such as the FDA Food Safety Modernization Act (FSMA) is designed to protect consumers by strengthening the food safety system. One aspect of this emphasis is reducing the presence of foreign materials found in food products. The first step in this process is to identify the materials and to discover their source. In the case of plastic and organic chemicals, infrared spectroscopy is a preferred technique for material identification and infrared microscopy is a common instrument to characterize small samples and particles. Since many of these contaminants are particles that are barely visible, FT-IR microscopy is a recommended way to quickly identify foreign materials in food products. In this presentation we will describe the use of a simple infrared microspectroscopy system to identify particles that can be isolated from a food matrix and also material trapped on a filter. We will discuss the benefits and tradeoffs of transmission, reflection and ATR methods as well as presenting example data that demonstrates different applications.
Recent developments in GC triple quadrupole MS technologies have allowed them to be recognised as being suitable in Europe for the control of maximum levels (MLs) of polychlorinated dibenzodioxins and polychlorinated dibenzofurans (PCDD/Fs) and dioxin-like polychlorinated biphenyls (dl-PCBs) in certain food and feed samples\textsuperscript{1}.

In the build up to regulatory changes in Europe, sensitivity was often the primary focus when demonstrating GC-MS/MS performance for this application. This is certainly important, but factors that allow confident (and regulatory compliant) confirmation of the presence of dioxins and dioxin-like compounds (e.g. ion ratios being within maximum limits) are very important considerations when evaluating instrument performance and method suitability for routine work.

In this study, limits of confirmation (LOC) of PCDD/Fs and dl-PCBs are assessed and applied to the measurement of food samples with concentrations around the European maximum limits\textsuperscript{2}. In addition, different methods of calculating instrument detection limits are discussed.

\textsuperscript{1} European Commission, Commission Regulation No 589, Off.J. Eur. Union, L164, 18–40, 2014

Keywords: Food Safety, Gas Chromatography/Mass Spectrometry, GC-MS, PCB's
Application Code: Food Safety
Methodology Code: Gas Chromatography/Mass Spectrometry
The invasive fungus Raffaelea lauricola is a biothreat killing avocado trees, Florida’s biggest tropical fruit crop. The resulting introduction of R. lauricola and now rapid spread of the fungus puts at risk commercial avocado groves in California, Mexico, and Central and South America. Due to the rapid spread of R. lauricola and the quick death of trees, early detection is essential. The only current method of pre-symptomatic identification is canine detection. Despite the high risk to food safety associated with biothreats and invasive species, canine detection use has been limited in this field. The lack of widespread application for canines in food safety targets is largely due to the lack of mimic training aids. In the case of R. lauricola, containment is difficult because fungal spores are easily spread. In order to create a mimic training aid, the volatile organic compounds (VOCs) in the headspace of infected trees must be fully characterized. The current study evaluated VOCs of inoculated young avocado trees in a greenhouse setting using solid phase microextraction-gas chromatography-mass spectrometry (SPME-GC-MS) in order to follow the progression of infection through VOCs in a controlled environment. Inoculated trees change their VOC production as part of their inherent defense system, slowing the production of certain categories of VOCs and producing others not seen in the healthy trees. These changes are detected before symptoms can be visually observed. The characterization of VOCs in this study will be essential in creating a mimic training aid for avocado trees infected with R. lauricola.
High-Throughput Chemical Analysis

Titration for Faster, Safer and Easier Analysis

Tight deadlines, high sample throughput, strict safety and quality standards – this is life in the analytical laboratory. This poster introduces the OMNIS titration platform that makes titration safer, faster and easier. We demonstrate the automation of four simultaneous analysis to increase sample throughput by 60% compared to existing methods. For safety, we describe a contact-free reagent exchange system that makes reagent exchange safer. Lastly, we show the safety and productivity benefits of this truly modular titration platform. Designed to grow with the needs of your laboratory, we present the OMNIS platform in expandable configurations that adjust to your laboratory demands.

Keywords: Automation, Electrodes, Environmental, Titration
Application Code: High-Throughput Chemical Analysis
Methodology Code: Physical Measurements
Use of superficially porous particles (SPPs) and sub-2 μm fully porous particles (FPPs) have shown tremendous improvements in efficiencies of achiral stationary phases (e.g. C18) over the last decade. Efficiencies of chiral stationary phases (CSPs) have traditionally lagged far behind in comparison due to multipoint interactions required for chiral discrimination and common use of 5 μm FPP supports. In this work, a variety of brush-type CSPs (oligosachharides, glycopeptides, anion exchange, pi-complex) were prepared using 2.7 μm SPPs to produce ultra-high efficiency chiral columns. Combined with modest backpressure and reduced retention of SPPs, these columns were used to perform ultrafast separation (< 60 s) on a large number of enantiomers in UHPLC and SFC with some eluting under a second. Wide scale applicability of these CSPs is demonstrated with use of different chromatographic modes and mobile phases. These high efficiency columns and resulting rapid separations can be used to monitor a process in real-time, serve as a fast second dimension column in 2D-LC, and increase throughput. Kinetic plots are used to match these new high efficiency columns with traditional commercial columns to provide an in-depth comparison.

**Keywords:** Chiral, Chromatography, HPLC Columns, Separation Sciences

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

**Methodology Code:** Liquid Chromatography
Abstract Text
Spectrophotometers are a universal tool for biological research as they provide a means to quantify and qualify biological materials of interest including nucleic acids, proteins, cell density, and many small molecules. However, limitations to the size of a traditional spectrophotometer limit its efficiency. To improve efficiency in these analyses, the capabilities of a spectrophotometer have been incorporated into the barrel of a micropipette. This micropipette spectrophotometer has several benefits over a traditional instrument such as relatively low manufacturing costs, small size, reduced to no sample loss and utility in enclosed environments. This instrument utilizes disposable tips like a traditional micropipette, 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 makes spectrophotometric analysis much more user friendly and efficient. The newest version of this instrument will be presented highlighting an improved tip design which makes it easy to use and attach to the micropipette during an analysis. Preliminary work utilizing snap-on radiation source modules will also be presented. Initial characterization of the device will be done using food dyes, and calibration curves will be completed for cell density and Bradford assay determinations.

Keywords: Biological Samples, Biospectroscopy, High Throughput Chemical Analysis, Spectrophotometry
Application Code: High-Throughput Chemical Analysis
Methodology Code: Portable Instruments
A new approach to building a paper based well-plate, here applied to cyanide detection is described. Chitosan encapsulated CdTe quantum dots with a maximum emission of 520 nm (CS-QD520) were synthesized, characterized and used as fluorophores. The chitosan-QD nanoparticle was specifically quenched by copper (II), and the quenched CS-QD520, was deposited onto a glass microfiber filter (GF/B) via electrostatic attraction. Subsequent introduction of cyanide ion (on sample application) resulted in fluorescence recovery upon release of copper from the QD surface to form copper (I) cyanide (CuCN), thus freeing the CS-QD nanoparticle. The “Signal-On” fluorescence linearly correlates to cyanide concentration in the range 0 to 200 uM. The QD fluorescent assay on GF/B was incorporated into a paper based well-plate format to enhance sample throughput. Three layers of paper well plate design were cut using a laser cutter and assembled using 5% polycaprolactone (PCL) in toluene and a low-cost laminator. The experimental conditions, namely probe concentration, amount of copper, and well-plate design, were optimized and applied to detect cyanide in drinking waters and environmental samples. The sensitivity of the assay dramatically increased since the reaction was preconcentrated and performed on the surface of the glass microfiber filter rather than in bulk solution (in the traditional well-plate format). The paper based well-plate format of the chitosan encapsulated quantum dot sensor makes rapid, high sample throughput, low-cost testing feasible and convenient for cyanide ion detection.

Keywords: Environmental/Water, Fluorescence, Lab-on-a-Chip/Microfluidics
Application Code: High-Throughput Chemical Analysis
Methodology Code: Microfluidics/Lab-on-a-Chip
Evaluating Mass Overload on Superficially Porous Particles

Introduction:
Superficially porous particles (SPP) are a powerful analytical tool for achieving fast LC analyses. The solid, impermeable core present in these particles increases the column efficiency by decreasing the diffusion path. However, the solid core also significantly reduces the surface area that is typically available in traditional fully porous materials (FPP). There is a potential concern that arises from a reduction in surface area: column loading ability. Column overloading (mass overload) occurs when the amount of material injected onto the column exceeds the available active sites of the stationary phase. The purpose of this study was to evaluate a series of analytes and determine the effects the solid core has on the loading ability of superficially porous particles.

Results:
The characteristics of the analyte significantly change the loading ability of a column. Overload on all of the columns tested for both the neutral and acidic analyte occurred between 1 and 10 µg on column while the basic analyte began to show overloaded conditions with as little as 0.01 µg on column. There is clearly a trend between the loading ability of the column and the superficial and fully porous particles, however, the trend does not appear to be related to particle volume alone.

Potentially the most noteworthy conclusion of this study is that the inherent efficiency of particles that are smaller and/or superficially porous makes the effect of overload conditions more pronounced. On a larger particle (i.e. a less efficient column), the initial w0.5 is substantially larger and an observed peak broadens to the point where the initial overload contribution is no longer distinguishable from the non-overload contribution. In contrast, the peak shape of a more efficient column cannot quickly recover from the initial overload which may result in the traditional peak shape often associated with mass overload.

Keywords: Chromatography, HPLC Columns, Liquid Chromatography, Separation Sciences
Application Code: General Interest
Methodology Code: Liquid Chromatography
The Potential of Under 250 nm Deep UV-LEDs in Chemical Analysis: 235 nm UV-LED Photometric Detection in Capillary Liquid Chromatography

In this work for the first time an LED in the spectral range under 250 nm is investigated as a light source for optical detection in chemical analysis. A 235 nm deep UV-light-emitting diode (deep UV-LED) is demonstrated in on-capillary photometric detection in capillary ion-exchange chromatography (IEC) for detection of common UV absorbing anions, here iodide, nitrate and nitrite. This investigation focused on fundamental properties of UV-LEDs, in particular emission spectra, radiometric power, effective heat dissipation with a passive heat sink, and energy conversion. The detection showed excellent linearity with stray light down to 0.6%, and effective pathlength at 92% of the used capillary inner diameter. The analytical performance parameters were demonstrated by detection of chromatographic separation of iodide in artificial seawater, showing limit of detection (LOD) of 1.30 µM, linear range 7.9 to 3937 µmol L⁻¹, and reproducibility as relative standard deviation (RSD) of peak height 0.6%, and peak area 0.7%. In addition, nitrite and nitrate were selected to study the potential of using deep UV-LEDs as light source in photometric detection for even lower wavelength absorbing analytes, showing reproducibility as RSD of peak height 1.2% and 3.6%, and peak area 0.9% and 2.9% respectively, and limit of detection 7 and 26 µmol L⁻¹. The LOD values compare favourably with literature values given the short detection pathlength used here (100 µm capillary ID) and the spectral overlap between the analytes’ absorption bands and the LED emission. The potential of deep UV-LEDs in optical detection for robust low cost portable devices depends on future advances for higher optical power and energy conversion LEDs.

**Keywords:** Detection, Instrumentation, Lab-on-a-Chip/Microfluidics, Liquid Chromatography

**Application Code:** General Interest

**Methodology Code:** Liquid Chromatography
A column packed with 2.6 μm or 2.7 μm superficially porous particle has been widely used on HPLC and UHPLC, because it showed not only excellent column efficiency but also lower back pressure than sub-2 μm column. Recently 2.0 μm and less than 2.0 μm superficially porous C18 columns were developed and have been available. In this study, 3 kinds of 2.0 μm and 1.7 μm superficially porous C18s and one totally porous hybrid C18, one totally porous monodisperse C18 were evaluated regarding efficiency, hydrogen bonding capacity, hydrophobicity, steric selectivity as well as peak shape of acidic, basic and metal chelating compounds. Compared C18 columns were SunShell C18 2 μm, Ascentis Express C18 2 μm, Kinetex C18 1.7 μm, Acquity BEH C18 1.7 μm and Titan C18 1.9 μm. Furthermore, efficiency loss due to frictional heat which yielded under high pressure and at high flow rate was observed. This efficiency loss was larger for a totally porous C18 than a superficially porous C18. Especially totally porous hybrid C18 showed the largest efficiency loss because of the lowest thermal conductivity.
High Performance Liquid Chromatography (HPLC) is one of the most widely-used analytical techniques in the world. Unfortunately, its operation almost always involves the use of hazardous solvents. In this paper, we investigate the use of food grade ethanol as an alternative mobile phase in reversed phase HPLC. Ethanol is renewable, non-toxic and environmentally benign, especially when compared to the predominant mobile phases in use today like methanol and acetonitrile (methyl cyanide). Ethanol mobile phase will also facilitate the use of HPLC in chemical-free environments, such as schools and seminar facilities. Environmentally friendly is one thing, but does ethanol work as a mobile phase? In order to properly answer that question, we had to develop criteria for judging mobile phase usefulness or “goodness.” We measured the column pressure in order to determine the relative viscosities, the UV absorption in order to determine the UV cutoffs, and the Van Deemter plots in order to determine the efficiency/speed profile of each solvent. We also measured some chemical properties including the selectivity (\( \alpha \)) in order to determine if the three solvents have differing chemistries and the retention factor (\( k \)) to determine their relative strengths as reversed phase solvents. We compared methanol, ethanol and acetonitrile on an Agilent 1200SL series HPLC with a Zorbax Eclipse Plus C18 column and diode array detector.

Keywords: Education, Environmental, High Throughput Chemical Analysis, HPLC
Application Code: General Interest
Methodology Code: Liquid Chromatography
Session Title: A Rugged C18 Stationary Phase for Accelerated Analysis

Date: Wednesday, March 08, 2017 - After

Room: Exposition Floor, Aisle 2500-2600

Abstract Text

Fast analysis used to be limited to chromatographers with access to expensive UPHLC systems. The re-introduction of superficially porous particle (SPP) liquid chromatography columns to the marketplace has given everyone access to the increased efficiency and peak capacity to decrease their analysis times. The high amount of hydrophobic retention of a traditional C18 phase is well established and every chromatographer includes one in their cache. This new SPP C18 is an endcapped Octadecylsilane phase for fast, rugged, consistent, and reliable reverse phase chromatography. The particles provide higher efficiency for faster analysis and increased sample throughput on typical LC systems. With them we achieve UHPLC like speed and performance with the 2.7µm SPP and higher efficiency per unit backpressure than traditional fully porous silica supports with the 5µm SPP. The versatility of these columns makes them ideal for separations in bioanalytical, CMC testing, food safety, environmental, and other testing areas. Example separations on these particles will be shown to illustrate their contributions to efficiency and overall sample throughput.

Keywords: Environmental, Food Safety, HPLC Columns, Liquid Chromatography

Application Code: General Interest

Methodology Code: Liquid Chromatography
The FluoroPhenyl stationary phase has long been marketed as a phase that offers alternative, or orthogonal, selectivity to a C18. The FluoroPhenyl phase offers unique selectivity by incorporating strongly electronegative fluorine atoms on a phenyl ring (Figure 1). In addition to the traditional reversed-phase dispersive interactions, this phase also exhibits shape selective, polar, cation-exchange and even HILIC retention mechanisms which aid in selectivity of specific analytes.

In this presentation we aim to demonstrate the useful and alternate retention of the FluoroPhenyl stationary phase. We chose several relevant target analytes which we plan to use to exemplify the unique retention characteristics of the FluoroPhenyl phase when used in either HILIC or reversed-phase mode. All of these analytes have been pursued due to either poor retention, poor resolution, or both on a traditional C18 phase.

Keywords: HPLC, HPLC Columns, Liquid Chromatography, Separation Sciences
Application Code: General Interest
Methodology Code: Liquid Chromatography
Influencing the Selectivity of Small Proteins and Peptides

With the influx of biotherapeutics in medical research and healthcare, the analysis of small proteins and peptides by liquid chromatography (LC) continues to grow. Many of these analyses utilize acid-modified mobile phases to improve peak shape; however, their effects on selectivity and retention are often not well understood. In this presentation, we will explore the effects of acid type and concentration, temperature, and gradient slope on the selectivity and retention of several peptide probes using the sterically protected superficially porous Raptor™ ARC-18 LC column (stable to pH 1).
Graphene is a single layer of graphite, it is a single atom-thick layer of carbon atoms, that are arranged in a flat, hexagonal lattice structure. The most common use was, for centuries, in pencil leads. However, graphene has important properties as: 200 times stronger than the strongest steel, high electrical conductivity, thin and light weight, high thermal conductivity and very high transparency. These superlative properties allows the use of graphene in semiconductor, electronics, battery energy and composites industries, and as touch screens in devices, water filtration systems, medial sensors and drug delivery. Elemental analysis is fundamental for the characterization of these type of materials to ascertain the quality. The FlashSmart CHNS/O Analyzer, based on the dynamic combustion of the sample, provides automatic and simultaneous CHNS determination in a single analysis run and the Oxygen determination by pyrolysis in a second run. To perform trace Sulfur determination the analyzer can be coupled with the flame photometric detector (FPD). This paper presents CHNS/O data of different graphene samples analyzed several times to show the repeatability obtained with the system.

**Keywords:** Characterization, Elemental Analysis, Materials Characterization

**Application Code:** Material Science

**Methodology Code:** Atomic Spectroscopy/Elemental Analysis
Carbon fibers are fibers about 5–10 micrometres in diameter and composed mostly of carbon atoms. The properties of carbon fibers, such as high stiffness, high tensile strength, low weight, high chemical resistance, high temperature tolerance and low thermal expansion, make them very popular in aerospace, civil engineering, military, and motorsports, along with other competition sports. Carbon fibers are usually combined with other materials to form a composite. When combined with a plastic resin and wound or molded it forms carbon-fiber-reinforced polymer (often referred to as carbon fiber) which has a very high strength-to-weight ratio, and is extremely rigid although somewhat brittle. However, carbon fibers are also composited with other materials, such as with graphite to form carbon-carbon composites, which have a very high heat tolerance. Elemental analysis can be very useful for the characterization of these type of materials to ascertain the quality, mainly for the determination of the high carbon content. While the nitrogen, hydrogen and oxygen content give information of the different thermal treatment of the fibers, and the residual content of hydrogen and oxygen are decisive for the good success or failure of the reaction of "densification" of the fibers. The FlashSmart CHNS/O Analyzer, based on the dynamic combustion of the sample, provides automatic and simultaneous CHNS determination in a single analysis run and the Oxygen determination by pyrolysis in a second run. This paper presents CHNS/O data of different carbon fiber samples analyzed several times to show the repeatability obtained with the
The reaction of several oil soluble phosphonium phosphate ionic liquids with iron substrates were investigated to determine their suitability as lubricant additives. The ionic liquids, dissolved in ester based lubricant base stocks were found to react with metal surfaces to form a phosphorus containing film. Scanning electron microscopy showed the deposition of a layer on the surface and x-ray fluorescence showed that the layer contained phosphorus. Infrared analysis of the film demonstrated the formation of poly phosphate chains on the material. Decomposition products from the ionic liquid were identified by GC-MS.
The carbonization of various types of metal-organic frameworks (MOFs) was carried out under inert gas flow and high temperature. The formation of carbonized MOFs (CMOFs) was monitored by Raman spectroscopy. In addition to the well-known D and G bands in the Raman spectra, the G' band feature was observed only in Fe- and Co-containing CMOFs. In contrast, CMOFs containing metals other than Fe and Co (Al, Cr, V, and Zr) do not show the G' band at all. The G' band was also observed by mixing the nitrate salts of Fe(III) and Co(III) with non-Fe- and Co-containing MOFs under the same experimental conditions as other CMOFs. The intensity of the G' band in carbon materials represents the degree of stacking order of carbon layers. It suggests that Fe and Co ions played important catalytic roles in the growth of ordered structures in CMOFs.
Porphyrin has a functionalized skeleton and its derivatives (e.g., zinc tetraphenylporphyrin; ZnTPP) are employed to make an active layer in organic photovoltaic devices. Since the device performance strongly depends on the molecular aggregation structure represented by the molecular orientation in the thin film, both analysis and control of the molecular anisotropic structure are of great importance.

ZnTPP is known to have a face-on or random orientation in an evaporated thin film. In addition, our previous study revealed that the spin-coated film prepared by using a 1,2,4-trichlorobenzene solution has a highly oriented structure, in which the porphyrin ring is nearly parallel to the substrate surface, that is, the face-on orientation. On the other hand, no studies have been reported on the edge-on orientation of ZnTPP. Therefore, a preparation of the edge-on oriented thin film has long been expected.

In the present study, the molecular structure of ZnTPP in an evaporated film has readily been controlled by chemically modifying a silicon surface using the self-assembled monolayer (SAM) technique. As a result, the edge-on orientation of ZnTPP in an evaporated thin film has first been realized on a high-density SAM of octadecyl trimethoxy silane covering a silicon substrate. The edge-on orientation was readily confirmed by using the IR pMAIRS technique.
Recently, quantum dots (QD) become major as fluorescent material for controllable and durable particles in many regions[1]. Among them, QD applied for wavelength shifting films that has high efficient conversion and high durability[2]. On the other hand, we proposed new concept of silicone optical technology (SOT) that is monolithic optical system using functional doping into polydi-methylsiloxane (PDMS) matrix. Black carbon pigment suppress internal scattering[3], Sudan dye molecules doping for absorption filter,[4] and SiC particle improved thermal conductivity. Since the dye molecule can be diffused even in the dry PDMS matrix, fixing dye-doped area needed barrier of SiO2 on the boundary. In this report, we report QD doping in PDMS as a tunable light source for optical analysis.

CdS QD (CS-460, NN Labs) was adopted as dopant absorbing around 460 nm and emit 490 nm. Since CdS affect the solidification process of addition polymerization, condensation type PDMS (KE-108, Shinetsu Chemicals) was used as matrix. The sample is 8x8 mm2 and 1mm thick, and doping concentration was limited up to 5mg/cm3. Though the large molecule of 2.6 nm length such as dis-bipenylfluorene can be diffused in PDMS[5], the QD of 4-4.5 nm diameter shows no diffusion. Since the nano colloid concentration isn’t so high, only 16% was converted into 470 nm. Thus, TiO2 particle (KE-COLOR-W, Shinetsu Chemicals) was co-doped to improve the efficiency by using random multiple scattering. The 6mm thick film with TiO2 doping showed better conversion efficiency.

In future plan, the combined structure of QD: PDMS with low TiO2 and PDMS with high TiO2 doping will be tried to optical excitation chamber for analysis application.


Keywords: Environmental Analysis, Fluorescence, Laser, Monitoring
Application Code: Material Science
Methodology Code: Fluorescence/Luminescence
Nanotechnology and nanoscience are important in various fields such as medical and materials engineering, electronics and biotechnology among others. Thus, there are ever increasing reports concerning the preparation and utilization of metallic nanoparticles (MNP) in the literature. It has proven challenging to develop approaches for the concentration and storage of metallic nanoparticles (MNP) because highly concentrated solutions of MNP are difficult to maintain in a stable dispersed state. They often agglomerate and/or aggregate during the extractive step or upon subsequent storage which alters their size and thus their properties which is undesirable. In this poster, we summarize our results on the utilization of a series of thermosresponsive zwitterionic surfactants, for the rapid one-pot synthesis with in-situ preconcentration of spherical monodispersed gold nanoparticles (AuNPs). The different experimental conditions that can impact the synthesis/preconcentration procedure are delineated. Under optimized conditions using the surfactant C[sub]8[/sub]APSO[sub]4[/sub], for instance, the AuNPs synthesized were wine red in color and exhibited their surface plasmon resonance transverse absorption band at 538.0 nm. The particles had a mean diameter of 15.6 +/- 5.1 nm as determined from TEM images. As calculated using Leff’s equation, the concentration of the AuNPs in the C[sub]8[/sub]APSO[sub]4[/sub] surfactant rich phase was 5.6 x 10 \[12\] particle / mL.
Effect of Magic Angle Spinning Rate on Deuterium NMR Spin-Lattice Relaxation of (Propylazanediyl)Bis(Methylene-d)Dibenzoic Acid Hydrochloride: Enhanced Spin-Lattice-Relaxation Due to Rotational Resonance

Deuterium NMR has been widely used in studying molecular motions in solids. At present, most of these studies are carried out under magic-angle spinning (MAS) of the solid sample, rather than static condition. Improved sensitivity is the main reason of using MAS. In addition, short spin-lattice relaxation times (T1) for rigid deuterons have been observed under MAS due to the presence of efficiently relaxing deuterons. In this study, we have prepared specifically deuterated solid compounds ( (propylazanediyl)bis(methylene)dibenzoic acid hydrochloride, di and mono deuterated at benzylic carbon) with the following structures: and characterized by deuterium MAS-NMR. Spinning rate dependent T1 of these compounds are presented and explained in terms of the solid-state structures.

Keywords: Magnetic Resonance, Materials Characterization

Application Code: General Interest

Methodology Code: Magnetic Resonance
Unraveling the Growth Mechanism of Perovskite Nanocrystals by Time-Dependent Spectroscopy Characterization

Due to their unmatched solar energy conversion efficiency that could reach up to 20%, the class of organic-inorganic hybrid materials known as perovskites has expanded over the past few years. The ability of these materials to redefine the field of photovoltaics has facilitated the development of numerous synthetic methods, however, little is known about their growth and formation kinetics in the colloidal state. Herein we report the synthesis of methylammonium lead bromide quantum platelets and quantum cubes. The ability to control the shape is a result of surface chemistry modification, reaction temperature, and finally the solvent system. Through this work it was determined that the critical parameters for the formation of quantum platelets were the presence of chlorinated solvent and a long chain amine. Where as the formation of the quantum cubes was kinetically driven. The optoelectronic properties of these materials were investigated using various spectroscopy techniques, in addition to surface and structural characterizations to confirm that the long chain amine is responsible for the ordered stacking of the quantum platelets. This research will help to improve both the design of synthetic methods to control shape and assembly of perovskite materials as well as to provide fundamental insight into their optoelectronic properties for the future optimization of solid-state devices.

Keywords: Energy, Materials Characterization, Nanotechnology, Surface Analysis
Application Code: Material Science
Methodology Code: Surface Analysis/Imaging
Molecular orientation is important in determining the physical properties of anisotropic materials. Raman spectroscopy supplies valuable information on molecular structure and when controlled polarization is added to the analysis it takes it one step further and provides information on molecular orientation. Polarized Raman spectroscopy can be used for the analysis of a wide range of materials ranging from crystalline solids, to aligned polymers, and even to engineered nano-materials such as aligned semi-conducting single walled carbon nanotubes (s-SWCNT). Polarized Raman imaging provides an efficient way to visualize molecular orientation in materials.

Keywords: Molecular Spectroscopy, Nanotechnology, Raman Spectroscopy, Vibrational Spectroscopy
Application Code: Material Science
Methodology Code: Molecular Spectroscopy
Most modern analytic techniques including scanning electron microscopy, X-ray photoelectron microscopy, and Raman microscopy generate complex datasets. Data with more than 2-dimensions is often referred to as hyperspectral data. The existing analysis techniques mostly center on principal component analysis (PCA) and multivariate curve resolution (MCR). However, the interpretation of results through these methods can be complicated, especially for the uninitiated user. Moreover, results generally depend on pre-processing of the data, which can further complicate the analysis. However, the most important problem with the existing methods is a lack of capturing of the variation in the entire dataset. Here, we present a revolutionary new approach to data processing. The DAHI algorithm is a direct data dimension reduction technique that is fast owing to the structure of the algorithm. Moreover, the method captures 100% of the variance in complex datasets, and it can identify spectral components even in trace quantities. The method has been developed in the Matlab coding environment, is based on very different mathematics than PCA and MCR, and is demonstrated on data sets from energy dispersive spectrometry, time of flight secondary ion mass spectrometry and Raman microscopy. The results indicate the effectiveness of the method compared to standard PCA and MCR analysis. Finally, we demonstrate the method on a hyperspectral image data of a human face that shows the effectiveness of the data reduction in terms of capturing all the information content.
The modification of inorganic surfaces has a profound impact in a number of important applications, including anti-corrosion, lubricating and anti-fouling surfaces. An often overlooked modifying agent for inorganic surfaces is siloxane polymers, due to the long-held belief that siloxanes are “unreactive”. However, the equilibration of siloxane polymers with acid/base functionalities of inorganic oxide surfaces provides a simple method for hydrophobization of these interfaces. Here, the reactions of liquid siloxane polymers with silica, alumina, titania and nickel oxide are characterized via ellipsometry, dynamic contact angles, and x-ray photoelectron spectroscopy (XPS). Additionally, the use of small molecule siloxane vapors as modifiers is also evaluated as a method for conformal coatings. One of the particularly interesting characteristics of these surfaces is their negligible contact angle hysteresis.
Microscopy

Holographic Characterization of Large Particle Contaminants in Chemical Mechanical Planarization Slurries

There is a need for detecting agglomerates of nanoparticles in chemical mechanical planarization (CMP) slurries in semiconductor processing. Traditional particle characterization techniques have difficulty measuring large particle contaminants in turbid media. Holographic characterization directly measures the size, refractive index, and three-dimensional position of each subvisible particle in CMP slurries, and rapidly establishes their concentration and size distribution. Based on holographic video microscopy and the Lorenz-Mie theory of light scattering, holographic characterization distinguishes agglomerates from nanoparticles and from other contaminants without requiring dilution or special sample preparation. We present measurement of agglomerate distributions in CMP slurries as a function of ionic strength, pH and mechanical stress.

Keywords: Characterization, Microscopy, Particle Size and Distribution, Semiconductor
Application Code: Quality/QA/QC
Methodology Code: Microscopy
Microscopy

Lipobeads’ Preparation and Imagining Using High Pressure Scanning Electron Microscopy

Since lipobeads are considered as the future platform for targeted drug delivery and controlled drug release, it is important to control structure and topology of the lipobeads and their constituents (microgels, liposomes) in the course of technological optimization of their synthesis. In this work, we use traditional high vacuum scanning electron microscopy (SEM) to characterize polymeric microgels, lipidic vesicles, and the result of their combination – micrometer sized lipobeads – at their hydrated state. It was discovered that these wet particles could be visualized without special sample preparation (staining, freezing, or metal coating). Using the high vacuum SEM, the difference in structures of microgels prepared by thermal and UV polymerization in different solvents was found. Three types of giant liposomes were recognized in conjunction with their size. SEM images of lipobeads’ structures were discussed for two methods for preparation of lipidic vesicles: lipid film gentle hydration and injection of alcohol solution of lipids into the hot water. The SEM imaging was shown to be a technique complementary to the optical microscopy, fluorescence microscopy, laser scanning confocal microscopy and atomic force microscopy for a quick characterization of the lipobeads’ structural organization. The results of this work also validate that the time for the scaled fabrication of lipobeads can be reduced from days to hours and, therefore, technological expenses on the production of lipobeads will not be a high cost for the gained advantages of their use.

Abstract Text

Since lipobeads are considered as the future platform for targeted drug delivery and controlled drug release, it is important to control structure and topology of the lipobeads and their constituents (microgels, liposomes) in the course of technological optimization of their synthesis. In this work, we use traditional high vacuum scanning electron microscopy (SEM) to characterize polymeric microgels, lipidic vesicles, and the result of their combination – micrometer sized lipobeads – at their hydrated state. It was discovered that these wet particles could be visualized without special sample preparation (staining, freezing, or metal coating). Using the high vacuum SEM, the difference in structures of microgels prepared by thermal and UV polymerization in different solvents was found. Three types of giant liposomes were recognized in conjunction with their size. SEM images of lipobeads’ structures were discussed for two methods for preparation of lipidic vesicles: lipid film gentle hydration and injection of alcohol solution of lipids into the hot water. The SEM imaging was shown to be a technique complementary to the optical microscopy, fluorescence microscopy, laser scanning confocal microscopy and atomic force microscopy for a quick characterization of the lipobeads’ structural organization. The results of this work also validate that the time for the scaled fabrication of lipobeads can be reduced from days to hours and, therefore, technological expenses on the production of lipobeads will not be a high cost for the gained advantages of their use.

Keywords: Biotechnology, Imaging, Microscopy, Nanotechnology

Application Code: Nanotechnology

Methodology Code: Microscopy
Microscopy

Imaging Dynamics of Single Cells During Adhesion, Migration, and Invasion

Cell adhesion is the complex process by which cells recognize and interact with surfaces that surround them. Biochemical techniques and theoretical modeling have shaped much of our current knowledge regarding this process, but optical microscopy studies have provided the most significant information on the mechanisms of cell adhesion. Recently, we developed a lateral microscope that enables the direct, label-free observation of cell-substrate interactions in real-time on any material, regardless of its composition, opacity, or topography. Because lateral microscopy provides a field of view orthogonal to those imaged by traditional optical microscopy, changes to cell morphology in the vertical direction can be observed, facilitating measurements of the contact angle between a cell and a substrate and the migration depth of a cell through extracellular matrix components. To exploit the benefits of this tool, we have developed assays that use rates of change in cell morphology to (i) characterize the expression of specific surface markers, (ii) describe the functional state of migratory and invasive cells, and (iii) correlate the expression of surface markers to cell phenotype. By quantifying the rates of change in morphology of single cells, as well as populations of cells, the various phenotypes that exist within a heterogeneous population—many of which are overlooked as statistical outliers—can be differentiated. The results of our approach will have significant impact on the study of wound healing, developmental processes, the immune response, and cancer metastasis.
In order to improve the bioavailability of chemically complex active pharmaceutical ingredients (APIs), amorphous solid dispersions are often preferred. However, amorphous APIs are typically metastable, with the potential to crystallize as a result of external environmental changes. Several techniques have been developed for detecting trace crystallinity such as powder X-ray diffraction (PXRD), differential scanning calorimetry (DSC), etc. Limits of detection (LoD) for these techniques are typically on the order of a few percent. Current efforts to improve LoD of PXRD by using bright synchrotron sources has pushed the LoD to about 0.2% crystallinity. However, this LoD may still be insufficient when the total drug loading of the drug formulation is itself only a few percent. Second harmonic generation (SHG) is a second order nonlinear optical process that is highly selective for noncentrosymmetric crystalline material. Because of the selectivity for noncentrosymmetric crystalline material, signal is only generated for crystals with negligible background from the amorphous matrix, making fast beam scanning SHG microscopy an excellent tool to detect trace crystallinity in amorphous materials. In close collaboration with GM/CA@APS at Argonne National Labs, we have developed an SHG-guided synchrotron PXRD system as a new method for the detection and characterization of trace crystallinity within an amorphous blend. Here, we demonstrate the use of this integrated SHG PXRD system for detecting a physical mixture of 100 parts per million ritonavir in hydroxypropyl methylcellulose. With this new method, we were able to lower the LoD to the ppm range and significantly decrease the measurement time.
Electronic cigarettes (e-cigs) have become a popular alternative to traditional cigarettes. E-cigs produce an aerosol when the e-liquid passes over the heated coil, vaporizes, and then condenses with water in the atmosphere. E-cig users have modified devices to drip e-liquid directly onto dry heated coils. The purpose of this study was to investigate two types of popular coils, Nichrome and Kanthal, used in e-cigs for metal composition pre- and post-heating. These coils contain toxic metals such as chromium, nickel, iron, and aluminum. Three different gauges of coils, 30, 32, and 34AWG, were evaluated by heating them up to approximately 1000°C at 3.7 and/or 4.3 V, 150 times. The elemental composition of the coils was determined using a Hitachi SU-70 Scanning Electron Microscope with, paired with EDAX Genesis Energy-dispersive X-ray Spectroscopy at 20 keV. Three measurements each were taken across the width of the center-most coil, with three replicates for each gauge. No significant difference in metal composition was determined between coils produced by the same manufacturer. Nichrome wires showed a significant decrease in nickel and iron from the surface of the wire. Kanthal wires showed a significant decrease in iron and chromium from the surface of the wire. ANOVA analyses showed statistically significant differences in composition between new and heated coils for all gauges and wire types. The direct application of e-liquids to a dry, heated coil may result in the aerosol containing toxic metals.

This research was supported the National Institute of Justice, Award 2014-R2-CX-K010

Keywords: Forensic Chemistry, Metals, Microscopy, Toxicology
Application Code: Other
Methodology Code: Microscopy
Total analysis time is composed of sample preparation, a chromatographic separation of the target compounds and column equilibration. It is possible that online SFE-SFC can be decreased the total analysis time because online SFE-SFC can be extracted the target compound and then successively separated with shorter analysis and equilibration time. The first is short extraction time because of no sample preparation. The second is high-resolution that isomers can be separated in a shorter time, and the third is short equilibration time. In this study, we examined improvement of total analytical work flow from sample preparation to column equilibration. As samples in the environment and food samples contain various matrices, sample preparation is needed before chromatographic analysis. The major sample preparations are extraction and clean-up, these are often time-consuming. In the case of online SFE-SFC, samples were loaded in the vessel and extracted with supercritical CO2 and a modifier. After extraction, target compounds were automatically transferred to SFC/MS with no human intervention. For SFE, target compounds can be extracted in several minutes. The extraction time was shorter than conventional methods.

We also demonstrated high-resolution separation and equilibration time using the SFC/UHPLC switching system. The system has two solvent-delivery pumps and one carbon dioxide delivery pump and can be used for SFC and HPLC with the single instrument. Switching analytical mode between SFC and HPLC was automatically performed in a single sequence. Here we will report the result compared with HPLC.

Keywords: SFC, SFE, Supercritical Fluid Chromatography
Application Code: Other
Methodology Code: Supercritical Fluid Chromatography
Supercritical Fluid Chromatography

Supercritical Fluid Extraction at 1000 Bar

Most supercritical CO2 extraction equipment and commercial production plants operate at pressures less than 500 bar because equipment costs increase significantly at higher pressures. This upper limit in pressure results in sub optimal extraction of many high value products. Results from supercritical fluid extraction experiments indicate that distinct solubility maxima are achieved between 650 – 1000 bar for many valuable compounds including: Sitosterol, Beta – Carotene, Cryptoxanthin, Lutein, Zeaxanthin and Astaxanthin.

Increasing supercritical CO2 extraction pressures up to 1000 bar may upgrade existing industrial processes operating at sub optimal conditions as well as lead to the discovery of effective supercritical conditions for new products.

This paper discusses examples of supercritical CO2 extractions at 1000 bar and newly designed equipment for laboratory experimentation and scale up at ultra-high pressures. Benefits include increased solubility of valuable compounds, reduced CO2 consumption, and significantly reduced cycle times resulting in cost savings.

Keywords: Biopharmaceutical, Isolation/Purification, Natural Products, SFE

Application Code: Food Science

Methodology Code: Separation Sciences
For several years, large amounts of research have been conducted in the search for a universal achiral SFC column. This universal column would be the SFC equivalent to the C18 column for reverse phase chromatography. These efforts have evaluated long lists of probes, many forms of column chemistry and employed sophisticated statistical treatment of screening data. However, to date there is still no magic universal column for SFC.

A universal column for achiral SFC applications would be convenient but does SFC need to have a universal column? Chiral chromatography in the pharmaceutical industry can be credited with shaping the entire SFC industry into its current position. Chiral chromatography does not have a universal column. SFC has excelled in this separation science niche because it is particularly effective at screening multiple columns and different eluent compositions. The same approach can be applied to achiral chromatography. SFC can be effectively applied to achiral applications when a limited set of columns are known to be applicable for the diverse range of compounds suitable for SFC.

The work presented here will discuss the development of achiral applications for SFC. The focus will be on compounds relevant to the pharmaceutical industry. Columns typically utilized for normal phase, reverse phase and chiral applications will be evaluated for use their applicability in achiral SFC applications. The effect on achiral selectivity due to changes in cosolvents and modifiers will also be addressed.

Keywords: Pharmaceutical, Supercritical Fluid Chromatography
Application Code: Phenomenex
Methodology Code: Supercritical Fluid Chromatography
Thermal Analysis

Calorimetry Studies of High Temperature Thermal Storage Materials used in Concentrated Solar Power (CSP) Systems

The thermal energy storage is a key issue for concentrated solar power plants. Molten salts are involved as heat transfer fluids in order to either directly transport the heat to the heat engine or to a heat storage system (for example Phase Change Materials). The thermal stability of commercially available molten nitrite/nitrates salt for solar thermal energy storage in different atmospheres was studied using TG/DSC-MS coupled technique. Additionally, the heat capacity of graphite-NaNO3 composite was accurately determined from 25°C to 450°C.

The specific heat of paraffin RT35 was measured with a SETARAM C80 calorimeter. The results will be presented and discussed.

Transposed temperature drop calorimetry method was applied to a composite ceramic-salt (SiO2/Na2SO4) for high-temperature thermal storage. The enthalpy change during melting were determined.

Keywords: Energy, Thermal Analysis
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Thermal Analysis

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Thermal Analysis

Thermal Analysis and Calorimetry in Process Safety Applications

Differential Scanning Calorimetry is the ideal thermal stability screening, small-scale (mg) tool for laboratories involved in process safety and thermal hazards investigations. The flexibility of such a technique allows the investigations with different experimental conditions to predict the various situations that can occur during a decomposition and to simulate the different parameters that can affect such phenomena.

Isothermal calorimetry: to measure heats of reaction and kinetic parameters of these reactions on a few grams of sample material. It requires longer tests, therefore isothermal calorimeters must have a signal that remains very stable over time like C80. Reaction calorimetry: To simulate a chemical process under conditions closer to the industrial ones, with larger volumes. Sensys DSC, C80 and DRC provide the accurate data that are needed for the evaluation of the safety of a given process, and the kinetics evaluation of these data with the AKTS software lead to the simulation of a variety of real-life conditions (kilo from to ton scale, and very long term behavior).

Keywords: Chemical, DSC, Process Control, Thermal Analysis

Application Code: Safety

Methodology Code: Thermal Analysis
Thermal Analysis and Calorimetry Applied to the Studies of 2D Carbon-Based Nanomaterials

Graphene, single-walled (SWCNTs) and multi-walled nanotubes (MWCNTs), onion-like carbons (OLCs) and nanodiamonds are attractive materials due to their two-dimensional structure, unique properties, and potential applications in many fields as electronics, catalysts, photonics, robotics, mechanics, energy storage, and orthopedics. All those new developments require a thorough study of the mechanical, physical and chemical properties of the nanocarbons and the corresponding composites. The density and the thermal expansion coefficients of MWCNTs-containing composites can be investigated using thermomechanical techniques. Thermogravimetry combined with differential scanning calorimetry (TG-DSC), is a powerful method to determine the amount of the impurities, the effect of the thermal treatment, and the thermal stability of CNTs and graphene composites. The isothermal immersion and oxidative calorimetry are commonly used to study the surface properties and thermodynamic stability of CNTs and OLCs, which are critical for their applications as catalysts and energy storage materials. These techniques will be introduced and illustrated by several examples on 2D nanomaterials.

Keywords: Nanotechnology, Temperature, Thermal Desorption, Thermal Analysis
Application Code: Nanotechnology
Methodology Code: Thermal Analysis
Evaluating and interpreting thermo-analytical measurement curves has always required both a certain level of experience and a certain expenditure of time. The user must decide how the curves should be evaluated and then manually perform the evaluation using tools provided by the analysis software. This can lead to variations in the analysis results and consume significant time. The recent introduction of automatic evaluation software for Differential Scanning Calorimetry (DSC) data has made the evaluation and interpretation of DSC data significantly easier, more reproducible and faster. For polymers, the automatic evaluation software searches systematically for all DSC effects like melting of the sample or melting of components (such as plasticizers and additives), glass transitions, crystallization, curing and evaporation. Any effects recognized are then automatically evaluated according to known standards; for example in the case of complex superimposed melting effects, several peak maxima are indicated.

New advances in software now enable the automatic analysis of Thermogravimetric Analysis (TGA) data. Any significant mass-loss steps (and mass gain steps) can now be evaluated automatically by the software through analysis of the DTG derivative curve. The DTG curve is also shown including automatically evaluated peak temperatures. The results yield evaluations that are not only fast and reproducible, but also purely objective since they are in no way affected by the user. Multiple curves can be analyzed simultaneously which provides additional time savings. The Auto Evaluation software is thus an enormous aid for beginners and also may serve as a valuable second opinion for experts.

Keywords: Software, Thermal Analysis
Application Code: Other
Methodology Code: Thermal Analysis
In determining what medications to send with astronauts to the International Space Station (ISS), NASA must consider how the different conditions, such as high-energy radiation exposure as well as differences in temperature, humidity, and CO2 levels on the ISS might affect the rate of drug degradation and the identities and amounts of the products of this degradation. In this study, sertraline (Zoloft®) tablets that had been stored on the ISS were investigated for potency and degradation products. Results were compared to those of sertraline tablets stored on earth at the Johnson Space Center (JSC) pharmacy. Potency and purity of all tablets were analyzed using HPLC methods from the United States Pharmacopoeia (USP); LC-MS methods were developed in order to identify and quantify degradation products. Preliminary results confirmed two degradation products present in ISS-stored tablets at higher levels than those stored at JSC; further testing is ongoing to determine the significance of this finding. The results of this research will be used to determine whether the sertraline tablets are potentially safe for long-term space missions.

Keywords: HPLC, Liquid Chromatography/Mass Spectroscopy
Application Code: Pharmaceutical
Methodology Code: Liquid Chromatography/Mass Spectrometry
As long-term space travel becomes more plausible, the potential degradation of medications in this environment has become an important safety question. Limited information is available about how the increased CO\(_2\), radiation, and microgravity on the International Space Station (ISS) and future spacecraft could affect the potency and safety of these medications. In this study, levofloxacin capsules that had been stored on the ISS were analyzed by high performance liquid chromatography (HPLC) and liquid chromatography-mass spectrometry (LC-MS). The results were compared to those of levofloxacin capsules stored terrestrially. Information from this study will help NASA to make informed decisions about what medications are suitable for future long-term space travel.
Investigation into the Stability and Potency of Ibuprofen Stored Aboard the International Space Station

Pharmaceutical storage is critical to the safety of astronauts living on the International Space Station (ISS). Medications undergo extensive terrestrial stability testing, as required by the US FDA, but little information is available on the stability and efficacy of commonly used drugs after exposure to space flight conditions. In this study, ibuprofen (IBP) tablets (400 mg) stored for 140 days on the ISS were investigated and compared to IBP tablets stored at the Johnson Space Center pharmacy. All IBP tablets were analyzed according to United States Pharmacopoeia (USP) methods (with deviations) to quantify both IBP and its known, regulated degradant 4-Isobutylacetophenone (4-IBAP) using high performance liquid chromatography (HPLC). In addition, liquid chromatography-mass spectrometry (LC-MS) methods were developed for identifying and quantifying other degradation products. Results for ISS and terrestrial IBP tablets will be presented, and the implications for storing ibuprofen tablets on future long-term space missions will be discussed.

Keywords: HPLC, Liquid Chromatography/Mass Spectroscopy, Pharmaceutical
Application Code: Pharmaceutical
Methodology Code: Liquid Chromatography
Degradation of drugs during storage can affect not only their efficacy but also their toxicity, depending on the degradation products that are formed. For this study, NASA provided samples of phenytoin extended release capsules that had been stored on the International Space Station (ISS). These were assessed for both potency and purity and results were compared to those of terrestrial samples. All samples were analyzed following United States Pharmacopoeia (USP) methods for phenytoin potency as well as degradants known as phenytoin related compounds A and B. In addition, liquid chromatography – mass spectrometry (LC-MS) methods were developed for identifying any other degradation products. LC-MS analysis indicated the formation of a novel degradant. These preliminary findings suggest that more testing of controls and ISS tablets is required to confirm chemical purity and drug safety for ISS crew members.

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

**Application Code:** Pharmaceutical

**Methodology Code:** Liquid Chromatography/Mass Spectrometry
As nanoparticles become increasingly commonplace in many consumer products, the release of nanomaterials into natural waters is inevitable and potentially harmful. Silver nanoparticles (Ag NPs), the most widely used type of nanomaterial, is of special concern due to the toxicity of silver to many aquatic organisms and recent studies have demonstrated the importance of nanoparticle surface chemistry in the behavior of Ag NPs such as dissolution and aggregation. However, to date these studies have not measured the degree to which surface adsorption occurs. We have investigated a method by which Ag NPs are allowed to react with various organic compounds and, following a size-based separation, the amount of compound that remains unadsorbed is determined by HPLC. The separation method proved to be unreliable for quantitative adsorption measurements, but results indicated adsorption of benzoic acid derivatives bearing hydroxyl, carboxylic acid and amine groups were negligible under the conditions examined. Therefore a method that did not require a separation step was adopted to examine the Ag NP surface interactions of thiols, which are known to have a high affinity for silver. The unadsorbed thiol concentration in solution can be measured directly using voltammetry without the need for separation. Preliminary work demonstrates the potential value of this method for the in situ determination of thiol adsorption by Ag NPs.
Providing novel media for separating industrial waste gases is important for decreasing the amount of unwanted gases that are emitted to the atmosphere. Two promising methods for gas separations are selective transport through mixed-matrix membranes (MMMs), and sorption by high surface area carbons (HSACs). Our objective was to learn more about the transport mechanism of gases in MMMs and the efficacy of HSACs for gas separations. Our MMMs consisted of 0, 5, 10, 20, or 30 wt.-% hollow carbon spheres embedded in a triblock copolymer membrane. CO\(_2\) and N\(_2\) permeability and selectivity of the MMMs was measured in a custom test chamber at 35, 50, 75, 100, and 200 kPa and 25 °C. MMM permeability increased with pressure, but failed to return to initial levels when retested at 35 kPa after testing at 200 kPa. Scanning electron microscopy showed changes in membrane morphology after testing.

We synthesized our HSACs by hydrothermally carbonizing three carbonated beverages – Mello Yello[registered], Mtn Dew[registered], and Push[registered] Orange Soda. After carbonization the materials were thermally treated at 1000 [degree]C under nitrogen. We achieved surface areas between 300 and 820 m\(^2\)/g by the Brunauer-Emmett-Teller method. Gravimetric analysis showed the samples adsorbed up to 13% CO\(_2\) by mass. Energy dispersive spectroscopy showed the samples to be [greater than]89% carbon, with trace amounts of heteroatoms such as phosphorus or sulfur.

**Keywords:** Membrane, Separation Sciences
**Application Code:** Environmental
**Methodology Code:** Separation Sciences
Gas separations are being studied as a way to limit greenhouse gas emissions. We focused on two types of separations; mesoporous carbon membranes filled with phosphonium anion functionalized ionic liquids (ILs), and adsorbents derived from carbonated beverages. We tested membranes with pure ILs, as well as mixtures of different ionic liquids. As a function of pressure, we tested the ability of CO$_2$ and N$_2$ to pass through the membranes, as well as how much CO$_2$ passes relative to N$_2$ (selectivity). As pressure increased, permeability of CO$_2$ and N$_2$ increased, as did the selectivity. We derived powdered adsorbents from four carbonated beverages (Coca-Cola®, Push Orange®, Diet Pepsi®, and Diet Mtn Dew®) and distilled water (30 mL) containing 1 gram of Splenda®. We also added 1 gram of Splenda® to each 30 mL sample of diet soda. We hydrothermally carbonized the samples at 200 [degree]C and then further carbonized them in a furnace at 1000 [degree]C. The products were porous, high surface area carbon powders that could adsorb CO$_2$. The powders were characterized through surface area measurements with a volumetric adsorptive analyzer, gas adsorption measurements with a gravimetric analyzer, scanning electron microscopy, and energy dispersive x-ray spectroscopy. The powders adsorbed more CO$_2$ than standard ordered mesoporous carbon and had moderately high selectivity values (~15), and high CO$_2$ interaction energies (up to 29.2 kJ/mole). Our research further developed two novel techniques to separate gas and reduce greenhouse gas emissions.

Keywords: Characterization, Energy, Environmental/Air, Separation Sciences

Application Code: Fuels, Energy and Petrochemical

Methodology Code: Separation Sciences
Carbon Quantum dots as nanosensors offer unique biochemical advantages over traditional metal-metalloid quantum dots, including low cytotoxicity, high temporal stability (months in a fridge), and resistance to degradation by extreme pH conditions. Furthermore, the high population of carbonyl groups (-C=O, -COOH) on the surface of the dot allows for easy modification such as linkage to ion sensitive dyes with the aid of a linking agent such as Fluorescein isothiocyanate or EDC (N-(3-Dimethylaminopropyl)-N-ethylcarbodiimide hydrochloride) combined with a dye. We will report on carbon quantum dots fabricated using a solution of citric acid in ethanol added to heated AEAPMS (CAS No. 3069-29-2) as the capping agent (surface passivator). pH sensitivity was enhanced through a linkage with naphthofluorescein dye, producing a pH probe with linear dynamic ranges of approximately pH 0-4 and pH 8-12.

Keywords: Bioanalytical, Fluorescence, Nanotechnology, Sensors
Application Code: Bioanalytical
Methodology Code: Sensors
Abstract Text

Oxidative DNA damage is one of the leading causes of cancer and other degenerative diseases, and Reactive Oxygen Species (ROS) (e.g., hydroxyl radical) are well associated with the formation of DNA lesions. One avenue to reduce the concentration of ROS and/or prevent the formation thereof is through the local availability of antioxidants. We are currently exploring the application of thione and selone complexes as potential antioxidants through mechanisms of action that have previously not been fully elucidated. The best known mechanism of DNA protection is radical scavenging, whereby an antioxidant reacts with ROS to form an unreactive product. In the current work, however, we are investigating a mechanism of protection in which the target antioxidant (i.e., a S or Se-containing compound) may coordinate with a physiologically relevant metal of interest (e.g., Fe[II]), thereby preventing the initial formation of ROS. As a model system for study, we are investigating compounds [N,N’]-dimethylimidazole thione (dmit) and selone (dmise), as well as methimazole (MetIm). Results from gel electrophoresis and polymerase chain reaction (PCR) demonstrate that these three compounds partially protect DNA from oxidative damage, as evidenced by the continued successful replication of DNA after exposure to ROS-generating species such as hydrogen peroxide. Further, by direct comparison, these species are found to protect DNA at lower concentrations than the biologically significant antioxidant glutathione, and demonstrate evidence of their efficacy through metal binding. Preliminary liquid chromatography data likewise shows evidence of partial protection of DNA by dmit, with additional compounds under investigation.

Keywords: Electrophoresis, HPLC, Liquid Chromatography/Mass Spectroscopy
Application Code: Bioanalytical
Methodology Code: Liquid Chromatography/Mass Spectrometry
Diverse cationic biocides are used in low (ppm) concentrations in personal care products such as multipurpose contact lens solutions (MPS) to inhibit bacterial growth. Characterization of these compounds is essential to understanding their efficacy, particularly for complex mixtures that incorporate a broad range of discrete forms. The biocide polyhexamethylene biguanide (PHMB) is incorporated as a polycationic, polydisperse additive in MPS and other sanitary solutions. Recently, we developed a method using ultra high performance liquid chromatography (UPLC) coupled with electrospray (+) mass spectrometry (ESI-MS) to elucidate the structures of multiple oligomers from commercially available PHMB, which comprise a significant size range. To better investigate the distribution of oligomeric species for purposes of ascertaining biological efficacy and contact lens adsorption, we have performed equilibrium dialysis using membranes of various sizes (MWCOs). By using multiple membranes followed by size exclusion chromatography (SEC) on dialyzed samples, we have successfully isolated PHMB samples in a range of molecular weights including 1kDa-2kDa and 3.5kDa-5kDa. This distribution is confirmed via dynamic light scattering (DLS). Re-injection and analysis of isolated samples via UPLC-MS provides a mechanism to correlate complex MS fragment analysis of PHMB oligomers with size estimation provided by SEC/DLS. PHMB masses in the 1kDa-2kDa retentate give masses of 184.1938, 284.4108, 342.3080, 367.3012, and 481.2949, representing 1-3 monomer units. The 3.5kDa-5kDa retentate samples include masses of 617.3708, 721.9464, 778.4417, and 961.5828, representing 3-6 monomer units.

Keywords: Bioanalytical, HPLC, Light Scattering, Liquid Chromatography/Mass Spectroscopy
Application Code: Bioanalytical
Methodology Code: Separation Sciences
Previously our lab has investigated the properties of a broad range of \([\text{Cr(diimine)}]_3^{3+}\) complexes in relation to their potential to behave as DNA photocleavage and/or photoadducting agents. Several possible binding modes have been proposed as a function of diimine structure/identity; for example, DNA intercalation of complexes containing the planar aromatic dipyridophenazine (DPPZ) ligand has been correlated with photoxidation of the guanine nucleobase. Recently, we have compiled evidence supporting the possibility of a covalent binding mode, in which Cr(III) complexes containing photolabile ligands (e.g., 1-methylimidazole, 1-Melmid) are shown to bind duplex DNA and custom DNA oligomers following exposure to UV irradiation at 350 nm. This is of significant interest for an octahedral transition metal complex in comparison to binding observed with the popular chemotherapeutic cisplatin (square planar), and the potential use of these Cr(III) complexes for photodynamic therapy (PDT). In the current work we investigate the characterization and quantitation of Cr:DNA adduct formation using Ultra-High Performance Liquid Chromatography (UPLC) coupled with Electrospray Mass Spectrometry (ESI-MS) as a function of photolysis conditions. In these studies we compare the ability of several Cr(III) diimine complexes including \([\text{Cr(diimine)}]_2(1-\text{Melmid})]_2^{3+}\) to bind to short, single-stranded DNA oligomers of varying nucleobase composition, with particular emphasis on thymidine content. Poly thymidine oligomers will be compared to oligomers lacking thymidine in order to confirm the specificity of the Cr(III) complexes. Adduct formation will be analyzed based on a reduction in unbound oligomer peak area and the appearance of novel Cr:DNA adduct species.
## Abstract

### Abstract Text

Water supplies can be easily contaminated by common pesticides, thus harm the environment and organisms that are exposed to it, and lead to numerous health defects to the human body. The four pesticides of our interest are atrazine, simazine, metolachlor, and cyanazine. This project involves a qualitative and quantitative analysis of these widely applied pesticides and their degradation products in various real-world samples (water, river and lake) with multiple separation/detection techniques, including Gas Chromatography-Mass Spectrometer (GC-MS) and High-Performance Liquid Chromatography (HPLC)-UV. DPX technique is used to pre-concentrate the water samples. The detection limit, quantitation limit, linearity and spike recovery from different techniques will be discussed.

### Keywords
- Chromatography
- Environmental Analysis
- GC-MS
- HPLC

### Application Code
- Environmental

### Methodology Code
- Liquid Chromatography

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### Session Title
Undergraduate Poster Session

### Abstract Title
Sensitive Pesticide Detection in Drinking Water and Georgia Lake Waters Using HPLC-UV

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Chiral silanes have become very important in the field of chemistry due to their use in stereoselective synthesis and organometallic chemistry. Silanes are also of particular interest in the pharmaceutical industry due to their potential drug delivery abilities. Because silanes can react differently based on their enantiomeric or diastereomeric ratio, separating and quantifying these compounds is important for these applications. Typically, chiral separations are performed via HPLC using cellulose-based columns, however the limited flexibility of this method prevents it from separating certain chiral silane compounds. Here, we develop a method to separate novel chiral silanes using chiral micellar electrokinetic chromatography. Successful chiral separations were acheived for two different chiral silanes, (menthoxy)(methyl)(napthyl)phenylsilane and (butyl)(menthoxy)(methyl)phenylsilane, using cyclodextrins and chiral surfactants as additives in a non-aqueous methanol solvent.
By utilizing an Interface between Two Immiscible Electrolyte Solutions (ITIES), one can monitor the transfer of ions across this interface with high temporal and spatial resolution. Pipet-supported ITIES probes can be used to detect both redox active and non-redox active neurotransmitters, such as acetylcholine, on a micro- or nano-scale. In this case we are presenting the use of ITIES nano-electrodes to detect and quantify the real-time release of acetylcholine, a non-redox active neurotransmitter, from the somata of individual live neuron cells.

In these experiments, pulled nanopipets, surface modified with silane and filled with organic solution, are submerged in the cell medium (artificial seawater) and function as a neurotransmitter-sensing electrode. When polarized, the ionic neurotransmitters transfer across the interface and thus are detected. These probes were placed directly above live Aplysia californica cells in culture which were stimulated with elevated-potassium solution; positioning and amperometric recordings were performed using Scanning Electrochemical Microscope (SECM).

The results show consistent, well-defined peaks detected in response to cell stimulation. Various control experiments were performed to verify that these peaks were due to cellular release. Kinetic and quantitative analysis of peaks indicate that we observed the summation of multiple release events. Further probe experiments show that the nano-electrode and experimental conditions were selective for acetylcholine detection over other possible transmitters. The nano-electrode has a limit of detection on the micromolar scale, a relatively broad dynamic linear range, and rapid detection times in comparison to other methods of acetylcholine detection. ITIES-nanoprobes are an excellent tool for the detection of non-redox active neurotransmitters from single live cells in real-time. These findings are significant for the study of neurotransmission in live cells.

Acknowledgement: Research was supported by the National Institutes of Health under award number R21NS085665. Authors also acknowledge Michelle L. Colombo and Jonathan V. Sweedler for their contribution to this research.
We present here the application of nano-resolved scanning electrochemical microscopy (SECM) using ion-sensitive probes to the study of neurotransmission in live neuronal cell cultures. Traditionally, the electrochemical detection of redox inactive neurotransmitters has been achieved indirectly using enzyme modified electrodes. Here we present a powerful alternative, where a nanopipet supported interface between two immiscible electrolyte solutions (ITIES) detects directly charged neurotransmitters without the need of electrode modification. Fabricated nanoscale pipettes contain a liquid-liquid interface. When polarized, the diffusion and transfer of ionic neurotransmitters across the ITIES generates a quantitative signal. Neurotransmitters such as dopamine, acetylcholine, tryptamine and serotonin each display distinct transfer potentials across the ITIES, making this tool specific to the species. (1, 2, 3) This enables their identification in complex systems using chronoamperometry and voltammetry. SECM coupled with nanopipet electrodes has provided unprecedented insight regarding chemical properties and dynamic processes occurring on the nanostructures. (4) We first place the electrochemical probe near the neuronal synapses. The probe subsequently detects neurotransmitter released upon stimulation of the neurons by a high concentration potassium solution. Control experiments with neurotransmitter propulsion towards the nano-interface confirmed that the signal pattern was consistent with that of acetylcholine. This is the first in-vivo demonstration of acetylcholine release dynamics at the single cell level.

(1) Analytical Methods, 2015, 7, 7095-7105.
(2) Analytical Chemistry, 2015, 87, 5095-5100.
(3) Journal of The Electrochemical Society, 2016, 163, H3072-H3076
(4) JACs, 2012, 134, 9856–9859.

Acknowledgement: This research was supported by the National Institutes of Health under Award Number R21NS085665.
Forensic laboratories are experiencing escalated workloads and limited resources as illicit drug use continues to increase in the United States. In addition, the complexity of sample matrices provides additional analytical challenges despite efforts in improving drug testing protocol. The objective in this study was to implement more effective methods for the analysis of drugs in urine. The preparation and analysis times were significantly reduced without sacrificing thorough characterization of sample constituents. Standard extraction techniques were used to prepare non-derivatized urine samples. These extracts were spiked with different classes of drugs and analyzed by a novel benchtop GC-TOFMS. Sample analysis throughput was improved by optimizing instrument acquisition parameters and software processing methods. Comprehensive data processing included automated peak finding, retention index calculations, quantitation, and spectral similarity searches using several databases. Various classes of compounds, including sterols, terpenes, illicit drugs and pharmaceuticals were detected in human urine. Good calibration linearity was obtained for opiates, barbiturates, and amphetamines.

**Keywords:** Drugs, Forensics, Gas Chromatography/Mass Spectrometry, Time of Flight MS

**Application Code:** Homeland Security/Forensics

**Methodology Code:** Gas Chromatography/Mass Spectrometry
In 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 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 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 an acceptable alternative in this instance. The work presented here was done using the sample preparation method from an ICP-MS analysis done by Gh. Tanase, et al., and the digested samples were then analyzed using a Perkin-Elmer Optima 5200DV 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:** Forensic Chemistry, ICP, ICP-MS, Plasma Emission (ICP/MIP/DCP/etc.)

**Application Code:** Homeland Security/Forensics

**Methodology Code:** Sampling and Sample Preparation
Zn has an important role in many physiological activities in the bloodstream, including binding to a large number of enzymes and proteins and enabling them to perform their biological functions. Here we look at the binding of Zn to human serum albumin (HSA), a large 66.5kDa globular protein that serves as a critical carrier protein in the bloodstream. Quantification of binding affinities between Zn and HSA were obtained using equilibrium dialysis and Scatchard analysis. Inductively coupled plasma optical emission spectroscopy (ICP-OES) was used for quantification of nanomolar metal concentrations of Zn. However, both the equilibrium dialysis and the ICP-OES methods require optimization to reduce the hindrance of a few notable issues. Nanomolar levels of Zn appear to adsorb to the dialysis membrane; adding 150 mM NaCl to a pH 7.4 Tris buffer solution counteracts this effect and allows the Zn to diffuse unhindered through the membrane. Commercially available rapid equilibrium dialysis (RED) devices are found to contain trace amounts of Zn, contaminating the sample and altering results. We developed a protocol to wash the device with the Tris-NaCl buffer, which reduced background Zn contamination below the limit-of-detection. The autosampler of the ICP-OES was modified to require a smaller volume of sample, while the replicate read time was optimized to improve sensitivity. After optimization of both the RED and ICP-OES techniques, we were able to determine a protocol for measuring the binding affinity between HSA and Zn. This will allow for future studies measuring the affinity between Zn and disease-modified forms of HSA.

**Keywords:** Atomic Emission Spectroscopy, Biopharmaceutical, Drug Discovery, Membrane

**Application Code:** Drug Discovery

**Methodology Code:** Atomic Spectroscopy/Elemental Analysis
This research focused on the characterization of the size and composition of indoor air pollution particulate matter. Airborne particulate matter is an air pollutant that is a large health concern. Particulate matter less than 2.5 microns (PM$_{2.5}$) can travel through the nasal passages and become embedded deep within the lungs. Exposure to PM$_{2.5}$ can increase during the usage of household products due to the fact that the emissions are contained within an indoor environment. Indoor air pollution from a stovetop, candles, and a hairdryer were observed in a 74.2 m$^2$ house. Additional experiments were conducted using a steam iron and a hairdryer to generate samples in a controlled 0.13 m$^3$ chamber. A scanning mobility particle sizer (SMPS) utilizing a differential mobility analyzer (DMA) and condensation particle counter (CPC) was used in order to obtain particle size distributions. Transmission electron microscopy (TEM) was used to visually analyze particle morphology, and energy dispersive x-ray spectroscopy (EDS) was used to determine particle composition. Various organic, ionic, and metal nanoparticles were present in the samples and they ranged in size from 13 to 573 nm. There was a large amount of PM$_{2.5}$ and ultrafine particles (< 100nm) present. The National Science Foundation and the Pennsylvania State University PSIEE Seed Grant funded this research.

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

Application Code: Environmental

Methodology Code: Physical Measurements
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 (SN). 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 (L-DOPA) 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. 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 the generation of hydrogen peroxide (H2O2) can serve as an indicator of the presence of oxidative stress. This experiment uses fast-scan cyclic voltammetry (FSCV) coupled with carbon-fiber microelectrodes to simultaneously monitor rapid, real-time, fluctuations of DA and H2O2 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.
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<td>Detection of Sulfated Polysaccharides Using Reversible Pulsed Chronopotentiometry with Polyion-Selective Electrodes</td>
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**Abstract Text**

Highly sulfated polysaccharides such as dextran sulfate and pentosan polysulfate are widely used as anticoagulants and therapeutic agents. Therefore, the detection of these polyanions is very important for biomedical applications. Classical potentiometry with polyion selective electrodes has been used for the detection of these polysaccharides. Even though it is simple and sensitive, this method is irreversible and limited to single use. Thus, in addition to being time and material consuming, this method is not convenient for continuous monitoring purposes. We present here a reversible pulsed chronopotentiometric polyion sensor for the detection of dextran sulfate and pentosan polysulfate. The polysaccharides have been measured directly using polyanion responsive electrodes and indirectly via titration with protamine using polycation responsive electrodes. In addition, the binding ratios of these polyanions with protamine have been determined.

**Keywords:** Biomedical, Electrochemistry

**Application Code:** Biomedical

**Methodology Code:** Electrochemistry
Chlorine dioxide (ClO₂) has been used for waste water treatment and as a disinfectant for sterilizing products including equipment for medical procedures and food processing. ClO₂ is a highly soluble gas that has strong oxidizing capability. It can therefore be used to degrade organic and inorganic chemical pollutants in the waste water as well as to reduce the levels of pathogens. It has been shown to denature and degrade proteins. The purpose of this research is to observe how quickly ClO₂ reacts with aromatic amino acids including tyrosine, tryptophan, and phenylalanine as well as to identify the byproducts of these reactions. In this research, the 25 ppm aromatic amino acids solution was exposed to 5.45 mM ClO₂ gas for 1 hour followed by the analysis of the amino acid byproducts using high performance liquid chromatography coupled to mass spectrometry (HPLC-MS) with electrospray ionization. After 1 hour of treatment, the levels of all three amino acids decreased. The degree of susceptibility to ClO₂ from the greatest to the least susceptibility are in the order of tryptophan > phenylalanine > tyrosine. The degree of susceptibility of these aromatic amino acids to ozone treatment was also studied with the goal of devising a treatment scheme involving both chlorine dioxide and ozone for deactivating the functions of peptides or proteins.
Heavy metal contamination in bodies of water is a major environmental concern, and the robust and inexpensive detection of these metals in solution is necessary to ensure the safety of drinking water, particularly in areas where access to standard testing methods is limited. Currently, the primary methods for the detection of heavy metals in solution are flame atomic absorption-spectroscopy (Flame-AAS) and inductively coupled plasma—both constrained to a laboratory setting. However, we propose an alternative, novel detection scheme for the detection of lead using a bacterial biosensor integrated into a microfluidic platform for quantitative field-analysis. [i] Caulobacter crescentus [/i] is a freshwater bacterium that exhibits a dimorphic life cycle that is advantageous in our detection scheme because of its ability to form robust hold-fasts on a microfluidic channel. As part of our preliminary work, we have investigated the relative localization and kinetics of lead ion uptake using Flame-AAS. After growing cells in lead-spiked media, we centrifuged, washed and resuspended in deionized water and analyzed the resulting solution with Flame-AAS. Results from these experiments indicate a multi-step uptake process that is time sensitive. Additionally, we have undertaken initial studies on the compatibility of [i] C. crescentus [/i] and a commercially-available, lead-specific fluorescent intracellular dye. Initial experiments suggest that the size of the dye limits cross-membrane permeability in viable cells. Future work on this project includes conducting more detailed temporal kinetic studies and further elucidation of dye-cell uptake activity. With more information, we will further evaluate the compatibility of [i] C. crescentus [/i] with our current proposed scheme.

Keywords: Biosensors, Environmental/Water, Fluorescence, Lab-on-a-Chip/Microfluidics
Application Code: Environmental
Methodology Code: Microfluidics/Lab-on-a-Chip
Recently, we demonstrated that siloxanes can be covalently bound to a variety of substrates through a simple-one step process. We deposited ultra-thin (~6 nm thick) films of polydimethylsiloxane (PDMS), fluoro-polysiloxane and amino polysiloxane on glass, silicon, alumina, and polyester surfaces. To further enhance the functionality of the films, we demonstrated incorporation of fluorescein in covalently bound polydimethylsiloxane (PDMS) films. This treatment was then accomplished in the presence of a quaternary ammonium salt -- tetramethyl ammonium -- which acted as a catalyst. The inclusion of the fluorescein-, rhodamine-, 9,10, diphenylanthracene, perylene-tetra-carboxylic-dianhydride, and amino-pyrene incorporated films provided robust ultra-thin fluorescent films on glass and silicon substrates. The emission maxima of the fluorescein-incorporated PDMS films was 514 nm, and they glow greenish in black light (UV radiation). In this poster, besides fluorescent-immobilized films, we also demonstrate grafting of a variety of siloxane polymers (namely poly-caprolactone, epoxy-cyclohexylethylmethylsiloxane, (mercaptopropyl) methylsiloxane-dimethylsiloxane, (carboxyalkyl)-terminated polydimethylsiloxane, vinyl-terminated-diphenylsiloxane-dimethylsiloxane) providing different functionalities to the siloxane films. The main aim of these studies is to enhance and tune properties of the covalently bound films through the grafting of the functionalized polymers or copolymers on the surfaces. In principle, antifouling, self-cleaning, self-healing, and antibacterial surfaces could be synthesized through this simple and facile method. We intend to use XPS and FTIR to characterize the nature of the covalently bound polymers, and cyclic voltammetry (CV) will provide us information on the quality and homogeneity of the functionalized films. Ellipsometry and goniometry will be used to measure the thickness and contact angle respectively of the grafted layers.

**Keywords:** Chemical, Fluorescence, Material Science, Polymers & Plastics

**Application Code:** Material Science

**Methodology Code:** Chemical Methods
The hanging drop cell culture has been utilized by many scientists as a viable and useful alternative to other cell culture techniques due to its ability to more closely replicate cell-cell interactions (1). Using synthesized and functionalized polymeric microscale particles, we aim to investigate the growth and harvesting of different cell types by forming an additional matrix to add on to the traditional hanging drop cell culture plate. Utilizing polymer particles as a 3-D scaffold, membrane binding proteins or cell-anchoring factors will be bound to the surface of the microparticles utilizing surface chemistry functionalization techniques. The functionalized product will be added to the hanging drop cell culture plates to mimic the external natural microenvironment. The expectation is that the functionalized micro particles will form a matrix that will aid in decreasing the size of the spheroids and cell clusters generated during culturing process within the droplets. By decreasing the size of the cell colonies, it is believed that more cells may be able to thrive in the culture due to imposing a restriction on the size of the spheroids thereby allowing much larger surface area to internal volume ratio. This, in turn should allow for key nutrients and waste products to diffuse to cells for healthy growth in an effective and facile manner into the replicated environment. This scaffolding technique may provide a viable alternative to 3D cell culturing that features a consolidation of the Hanging Cell Culture and Electrospun Fibre Culture techniques in order to better target and grow a more diverse population of cells.

References
In contrast to cicadas which emerge annually, periodical cicadas are unique in that they have either a thirteen or seventeen-year emergence cycle. Because these organisms spend the majority of their lifetime underground feeding on the xylem of trees and do not travel large distances as an emerged adult, they are well suited to represent the elemental characteristics of the soil at a specific location. In this work, Brood V periodical cicadas, a seventeen-year cicada native to Ohio, West Virginia, and Pennsylvania, were collected in May 2016. Cicadas were dried to a constant mass, dissolved in nitric acid and hydrogen peroxide using microwave-assisted digestion and elemental profiles for each sample were determined using Inductively Coupled Plasma-Atomic Emission Spectrometry and Inductively Coupled Plasma-Mass Spectrometry. Differences in the trace metal levels in cicadas collected from a variety of locations in the emergence area will be discussed.

Keywords: Atomic Emission Spectroscopy, Environmental, ICP, ICP-MS
Application Code: Environmental
Methodology Code: Atomic Spectroscopy/Elemental Analysis
Undergraduate Poster Session

Abstract Title: Mathematical Strategies for Identifying Cremated Remains

Abstract Text:

Over a decade ago, the Tri-State Crematorium in Noble, GA was investigated for improper disposal of deceased individuals. At the conclusion of the investigation, authorities found that families were given cement powder in place of the authentic cremated remains. Because cremated remains are visually indistinguishable from a variety of other materials, many studies have examined instrumental methods for authenticating human cremated remains. In this work, Inductively Coupled Plasma-Atomic Emission spectrometry was employed to determine the trace metal content of human cremated remains and a variety of other samples (e.g. cement, grout, dirt, sand, fertilizer, and animal cremated remains). Trace metals having the most variation across all samples were plotted two-dimensionally and used to distinguish authentic human cremated remains from other materials. Principal component analysis of the elemental information was also utilized and more clearly established the authenticity of a cremated remains sample.

Keywords: Atomic Emission Spectroscopy, Bioanalytical, Chemometrics, ICP

Application Code: Bioanalytical

Methodology Code: Chemometrics
Diazonium ions have been used to modify graphitic carbon for a variety of uses, including as electrodes and LC stationary phases. The grafting is presumed to occur via reduction resulting in a radical that forms a covalent bond to the carbon surface. To our knowledge, there are no literature reports of diazonium ion grafting of graphitic carbon particles for use as solid phase extraction (SPE) media.

The aim of this work was to modify commercial carbon SPE media with carboxylic acid groups and test the effectiveness for preconcentration of copper and lead ions. Diazonium ions were synthesized from 4-aminobenzoic acid and 4-aminosalicylic acid in the presence of nonporous graphitic carbon particles, comparing the reactions with and without sodium borohydride as a chemical reducing agent. SPE tubes were packed with the carbon particles and used to preconcentrate solutions of copper(II) and lead(II) ions prior to atomic absorption measurements.

Low absorbance values resulted from buffered solutions of lead or copper ions that had been passed through the modified carbon, indicating that the carbon was effectively trapping these ions. Absorbance values for acidic eluent solution showed 50-90% recovery of the ions depending on conditions. Best recovery values were obtained for copper ions with tubes packed with carbon that had been reacted with carboxybenzenediazonium ions using sodium borohydride. Salicylate-modified tubes required a higher acid concentration for efficient elution of copper. Lead ion preconcentration was not improved by modifying the carbon with carboxylic acid groups.

Keywords: Atomic Absorption, Lead, Solid Phase Extraction
Application Code: Environmental
Methodology Code: Sampling and Sample Preparation
Surface-enhanced Raman spectroscopy, SERS, is expected to play an increasingly important role in the field of analytical sciences. As is well-known, Raman spectroscopy is one of the few analytical techniques capable of giving information on the chemical structures without the need to place a sample in a vacuum. Coupled with the enhancement effect due to noble metal nanostructures, SERS promises to become a natural choice for on-site inspections.

Crucial to SERS is fabrication of requisite noble metal nanostructures. Our group is attempting to exploit various techniques. One method is based on random-MFON whereby randomly adsorbed SiO2 nanospheres are coated with a noble metal. In another method, surface-adsorbed base metal nanoparticles are used as seeds for growing silver dendrites in a AgNO3 solution. Both methods give rise to SERS plates that are currently extensively evaluated.

Here, we report on a third method. Many biological systems are characterized by intricate nanostructures. We found that coating of butterfly scale with silver gives rise to a surface capable of showing the SERS effect. While the SERS signal intensity tends to be uniform within a single scale, the wing of a single species is composed of scales with different nanostructures, with the consequence that some scales are better suited than others. By further extending our study to numerous other species, we hope to find a structure that is particularly suited for SERS, serving as a model for fully-artificial systems.

Keywords: Bioanalytical, Nanotechnology, Pesticides, Surface Enhanced Raman Spectroscopy
Application Code: Bioanalytical
Methodology Code: Vibrational Spectroscopy
**Abstract Text**
Detection of biomolecular interactions is of paramount importance in analytical sciences, for example, for antigen-antibody reactions for POCT applications. In this presentation, we describe a localized surface plasmon resonance (LSPR) sensor based on optical properties of cap-shaped gold nanoparticles.

We have prepared our samples by first forming a randomly adsorbed monolayer of monodisperse SiO2 nanospheres on a glass slide with a 20 nm thick gold coating and then vacuum-depositing a thin layer of gold, 5 to 35 nm in thickness. These samples are characterized by a peak in the near-IR in addition to the usual visible peak. The near-IR peak has refractive index dependence greater than 500 nm/RIU, some four times better than the visible peak. Beside the film thickness and sphere diameter, the sphere adsorption density was found to dictate the peak wavelength.

While the improved sensitivity is very encouraging, we found a number of technical challenges. For example, use of the sensor in environments containing NaCl lead to an overall drift in the peak wavelength in the absence of any obvious biomolecular interaction on the surface; it could be attributed to change in nanoparticle morphology. We have solved this problem by coating gold nanoparticles with various thiol layers. We also had to take into account of absorption of near-IR above 1350 nm by water by minimizing the optical path length; this can be facilitated by use of a Fresnel lens with a periodically slanted surface. It is hoped our LSPR sensors can monitor biomolecular interactions under realistic environment.

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

**Application Code:** Bioanalytical

**Methodology Code:** Sensors
Unique bacterial siderophore chelate Desferrioxamine B (DFB)–modified acrylic polymer resin materials were synthesized and their uranium (VI) ion adsorption properties determined. The different polymeric particles were used as mother based materials. These resins were spherical and different micrometer sized particle (<7, 7–12 and 45–90 μm) which have individually different functional groups. The resin showed good uranium (VI) adsorption capability in the more neutral to weak alkali pH regions. For example, 0 to 100% uranium (II) ion recoveries from very weak alkali aqueous solutions were obtained using the DFB-modified resin. An adsorption capacity was investigated for these resins. In addition, the investigation of adsorbability of 52 elements on this resin was conducted. There resin could be expected applying to a separation column, a metal remover and an enhancement for sensitivity etc.

Keywords: Extraction, ICP-MS, Nuclear Analytical Applications, Solid Phase Extraction
Application Code: Nuclear
Methodology Code: Separation Sciences
With the usage of electronic cigarettes (e-cigs) on the rise, there is concern for the possible production of carbonyl compounds, which can be toxic, due to the decomposition of the e-juices. The most common nicotine solvents used in e-juices, glycerol and propylene glycol, have both been found to decompose into carcinogenic compounds, such as formaldehyde and acetaldehyde. The aim of this research is to determine if different e-juices to produce formaldehyde and to determine the amount of formaldehyde produced. The amount of formaldehyde was quantitated using the Hanzstch reaction, a colorimetric method that involves the addition of acetylacetone and ammonium salt to solutions containing formaldehyde. A yellow color is produced due the formation of diacetyllydihydrolutidine (DDL), which is indicative of the presence of formaldehyde in the solution. The formaldehyde content of the solution was determined by ultraviolet-visible (UV-VIS) spectrometry.

Keywords: Quantitative, Spectrophotometry, UV-VIS Absorbance/Luminescence

Application Code: Consumer Products

Methodology Code: UV/VIS
Hot electrons generated by excitation of localized surface plasmon resonances in nanostructured Ag substrates have been shown to reduce organic molecules directly adsorbed to the substrates. In this research project, we study the photoreduction of adsorbed 4-nitrobenzenethiol (4-NBT) to 4-aminobenzenethiol (4-ABT). This reaction can be followed using surface-enhanced Raman spectroscopy (SERS) because the spectra generated by 4-NBT and 4-ABT are distinct. The sensitivity of SERS allows the reaction to be monitored on the time scale of 1 second. In this poster, we report three primary results. In the first, we have used atomic layer deposition to create well-defined spacing layers of an insulator (Al2O3) between plasmonic particles (Ag nanoparticles) and a 4-NBT monolayer. We show that the reaction rate decreases as the spacing between the 4-NBT and the Ag increases, and compare our results to those in the literature. Second, we have studied the photoreduction behavior of 4-NBT monolayers adsorbed to two different types of Ag nanoparticles. The first is a common preparation using the reduction of silver nitrate by sodium citrate. The second is a solution phase Ag coating of silica beads. The second type of Ag substrate displays a more uniform morphology and therefore displays a more consistent correlation between SERS intensity and rate of the photoreduction reaction than that observed in previous research. Finally, we explored the photoreduction effect of a red (785 nm) laser excitation source vs. a green (532 nm) source. The photoreduction rate is much slower under red excitation. This allows the red laser to act as a SERS probe that can generate spectra of the adsorbed molecules independently of the photoreduction reaction.
# Size Reduction Thresholds in Paper-Based Analytical Devices ([\textit{micro}PADs])

**Background:** Paper-based analytical devices, also known as \textit{micro}PADs ([\textit{micro}PADs], are emerging as cost-effective, reliable, and portable biochemical assays. Fabricated via wax printing, [\textit{micro}PADs utilize capillary action to wick small amounts of fluid through lateral in-series test zones and provide a qualitative assessment of the analyte of interest. While [\textit{micro}PAD size is limited by the imprecise nature of the wax printing process, device area may be further reduced in the presence of an oxidizing agent. The purpose of this investigation is to determine the size reduction thresholds of [\textit{micro}PADs in aqueous sodium periodate (NaIO$_4$]).

**Methods:** Standard 20.25cm$^2$ [\textit{micro}PADs were saturated in varying concentrations of NaIO$_4$(aq.) for times ranging between 9 and 96 hours. Minimum hydrophilic channels, fluid required for wicking, wicking speed, and horseradish peroxidase (HRP) stability were tested. Miniaturized [\textit{micro}PADs were viewed under the scanning electron microscope.

**Results:** Saturation in 0.5M NaIO$_4$(aq.) for 48 hours provided optimal size reduction. When compared to standard [\textit{micro}PADs, miniaturized [\textit{micro}PADs displayed significant percent reductions in: area (~78%), hydrophilic channel width (~49%), and wicking velocity (~75%). HRP activity was stabilized for ~7 days longer on mini-[\textit{micro}PADs.

**Conclusion:** [\textit{micro}PAD parameters were significantly reduced after saturation in NaIO$_4$(aq.)]. Wicking velocity was significantly less, possibly on account of condensed cellulose fibers observed in SEM images, and may allow for increased test sensitivity by allowing samples more time to move through in-series test zones. Furthermore, miniaturized [\textit{micro}PADs increased enzyme stability, which could lead to a longer shelf life. Ultimately, miniaturized [\textit{micro}PADs may enable healthcare professionals to design ultra-portable devices requiring minuscule sample sizes.

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

**Application Code:** Biomedical

**Methodology Code:** Microfluidics/Lab-on-a-Chip
With the discovery of circulating microRNAs (miRNAs) in body fluids, an increasing number of studies have suggested their use as non-invasive biomarkers and as therapeutic targets for many diseases, in particular cancers. MiRNAs are single-stranded, noncoding 18-25 nucleotide RNAs that help regulate gene expression. Circulating miRNAs serve as ideal biomarkers for the prognosis of cancer due to its role in gene silencing and gene expression. Importantly, cancer-associated miRNA biomarkers can be detected via biological fluids thus allowing for a less-invasive form of monitoring. With 8.2 million people dying each year from cancer, optimizing the diagnosis of this disease is crucial for early prevention, but the success strongly relies on how efficiently the circulating miRNAs could be removed for detection by PCR or other assays. To improve the extraction efficiency of RNAs from biofluids, we have fabricated small metal oxide nanofiber polymers that have potential to extract miRNA, small RNAs, and DNA from biological and environmental samples more efficiently than current methods such as TRIzol and silica based extractions. Previous work has shown successful levels of miRNA in water-based extractions; therefore the current work has been focused on modifying the buffers to improve miRNA recovery from biological samples. After carefully optimizing the extraction and washing conditions more than 60% of the spiked miRNA could be recovered from serum, proving the preliminary success of the revised buffer recipes. Reproducibility tests are undergoing to ensure good consistency of our technique, and then it will be applied to extraction endogenous miRNA from serum and plasma for downstream identification and quantification, helping to improve cancer diagnosis using miRNA markers.

**Keywords:** Adsorption, Bioanalytical, Detection, Nucleic Acids
**Application Code:** Bioanalytical
**Methodology Code:** Separation Sciences
Self-assembled monolayers (SAMs) have the capability of altering the physical and chemical properties of a given substrate. SAMs may layer on a substrate in a variety of conformations, including island aggregates or complete monolayer coverage to optimize the altered properties of the substrate. Titanium and titanium aluminum vanadium (Ti-6Al-4V) were modified using mixed SAMs composed of two varying head groups, octadecylphosphonic acid (ODPA) and stearic acid (ODCA). ODPA easily attaches to substrates while ODCA is available in numerous reactive functionalities. In order to determine the attachment to the surface, diffuse reflectance infrared Fourier transform spectroscopy (DRIFT) was utilized to analyze the binding of both head groups. Matrix assisted laser desorption/ionization time of flight (MALDI-ToF) spectroscopy determined the presence of multilayers on the surface. Atomic force microscopy (AFM) was used to determine the homogeneity of the surface by comparing the modified and unmodified surface roughness values. ODPA was found to promote the binding of ODCA to the surface of both the substrates.

Keywords: Atomic Force Microscopy (AFM), FTIR, Surface Analysis, Time of Flight MS
Application Code: Material Science
Methodology Code: Surface Analysis/Imaging
American consumers spend upwards of $60 billion annually on their pets and expect quality pet food products, therefore knowing and controlling what is in pet food is an important aspect of this industry. A wide variety of dog (n=31) and cat (n=17) food samples were analyzed using Laser Induced Breakdown Spectroscopy (LIBS) and Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) for this project. ICP-OES requires extensive sample preparation but provides accurate elemental content. LIBS, on the other hand, involves no sample preparation but produces complex spectra with minimal quantitative information. Chemometric methods, including data preprocessing, principal component analysis, and modeling, were applied to these spectral data sets using Solo by Eigenvector Research, Inc. Commercial lab results for select samples were also used for comparisons. Goals of this work are to utilize the LIBS data to create a predictive model for elemental content and to discriminate between cat and dog food.
Samarium-cobalt magnets are rare earth magnets often used in engineering and consumer electronics due to their extreme magnetic strength, and ability to retain magnetism at high temperatures. The demand of these magnets is increasing as technology continues to advance, but traditional extraction of samarium from bastnaesite and monazite minerals is costly and produces large amounts of waste. Traditional methods for separation of rare earth metals include harsh organic solvents and concentrated mineral acids. Recently, ionic liquids have been used to separate samarium from cobalt, however, the high viscosity of these solvents contribute to difficulties during the extraction process. Water-immiscible deep eutectic solvents (DESs) are a new class of solvents that emerged from the ionic liquid field. These solvents are used in several processes such as catalysis, separations, organic synthesis, electrochemistry, and nanochemistry. In this study, hydrophobic DESs mixtures of tetraoctylammonium bromide and carboxylic acids, including oleic acid and lauric acid were synthesized. These DESs were mixed with water to observe the hydrophobicity. Subsequently, each DES was then mixed with aqueous samarium(III) solution, as well as samarium-cobalt magnet digest. The aqueous samples before and after extraction were analyzed for cobalt and samarium content using ICP-OES.
Undergraduate Poster Session

Analysis of Manganese Oxides Recovered from Abandoned Mine Drainage Passive Treatment Sites

Western Pennsylvania has been living with the after-effects of the coal mining industry for hundreds of years which leaves abandoned mine drainage to contaminate surrounding streams. The Slippery Rock Watershed coalition has been treating abandoned mine drainage for over twenty years using passive treatment systems. During the process of treating the streams, however, there are metal precipitates that are left as a waste sludge. Included in the sludge is manganese, which has applications in water treatment, batteries, and catalysis. We have tested the properties of manganese oxides recovered from these passive treatment sites and compared properties to commercially available manganese oxides. Results suggest the recovered manganese oxides have catalytic abilities, much like commercial manganese. The results were obtained by measuring the amount of oxygen evolved in the decomposition reaction with hydrogen peroxide. SEM-EDX, LIBS, and ICP-AES are used to analyze the surface and chemical composition of the recovered manganese in order to help explain the catalytic properties seen in earlier tests.

Keywords: Environmental, Environmental/Waste/Sludge, Plasma Emission (ICP/MIP/DCP/etc.), Surface Analysis
Application Code: Environmental
Methodology Code: Surface Analysis/Imaging
Biofilms are prevalent in multiple life settings, impacting society and the environment both positively and negatively. In the medical field specifically, infections are problematic. One of the first stages of biofilm development is adhesion of bacterial cells to surfaces. Popular biofilm prevention research focuses on this first stage by investigating the properties and effects of antimicrobial surfaces. Engineering surfaces that discourage bacterial adhesion is one technique in preventing biofilm formation onto abiotic surfaces. In this work, adhesion experiments using Pseudomonas aeruginosa were conducted to investigate the effects of a hydrophobic liquid infused BMA-EDMA polymer-coated microscope slide compared to an uncoated microscope slide. However, it is often difficult to quantify bacteria experiment results. Therefore, this research not only analyzes the effects of an antimicrobial coating, but also investigates the use of fluorescence microscopy and ImageJ software as a quantification technique to analyze adhesion results over time.

**Abstract Text**

**Keywords:** Bioanalytical, Fluorescence, FTIR, Surface Analysis

**Application Code:** Bioanalytical

**Methodology Code:** Fluorescence/Luminescence
With growing need for energy solutions, renewable, green energy must be developed as a viable source for energy in the near future. One application of this can be seen in residential situations, specifically those in which smaller living spaces are being utilized. The most notable place where solar power can be seen in this minimalistic residential setting is within the emergence of tiny homes. By establishing a minimalistic lifestyle, as well as drawing power from photovoltaic cells, we can greatly reduce our environmental footprint.

A large variety of solar cell types are currently available for consumers; however, narrowing down the options can be extremely difficult due to benefits of each. This research looks at comparing some of the major, commercially available solar cells. Efficiency testing was conducted on all cells as well tests to estimate longevity and durability of the cells, and testing was conducted to determine the chemical makeup and surface features of each cell type. The Tiny House Project at Westminster College, which will be used as a model for the research. This research will be used to design the solar system for the house the effort to show green building and living practices.

**Keywords:** Energy, Environmental, Surface Analysis

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

**Methodology Code:** Physical Measurements
Environmental awareness of heavy metal contamination in soil has increased in the last few decades, especially due to their potential bioaccumulation in livestock used for human consumption. Some heavy metals may be present in nutrient-supplemented feed such as corn silage, hay, grain, and haylage and can be detected in animal excretions, such as manure. The aim of our study was to determine the presence and concentration of selected heavy metal content in cattle, horse, and pig manure correlated with certain feeds in several northwestern Pennsylvania farms. We analyzed these manure and feed samples for copper (Cu), zinc (Zn), nickel (Ni), cadmium (Cd), and lead (Pb). We hypothesized that Cu and Zn, in particular, may accumulate at higher concentrations in manure due to the addition of supplemental nutrients into feed for growth purposes. Microwave induced acid digestion and inductively coupled plasma optical emission spectrometry (ICP-OES) methods were used to analyze the samples collected. In addition, we conducted a survey to collect information on general characteristics of the farms sampled. Results of the analysis showed that Zn levels in pig grain feed samples were significantly higher than other observed concentrations. In addition, increased amounts of Ni were found in manure but not in the feed, which suggests the environment may play a key role in the addition of Ni into the manure/soil. Our data showed that Zn and Cu metal concentrations were highest among livestock feed samples but not their manure, suggesting that the animal, via normal metabolic processes, may readily absorb these two essential elements. Although we did not find significant levels of harmful heavy metals, such as Pb and Cd, our analysis protocol could be used in future studies to analyze biological samples near areas of high pollution impact, such as gas extraction sites.
**Abstract Title**
The Efficacy of Using Liquid Foundation as a Primary Source of UV Protection

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**Co-Author(s)**
Gregg Gould, Kimberly Woznack

**Abstract Text**
Some liquid foundation cosmetics now advertise a sun protection feature. However, not many studies have been published on the effectiveness of liquid foundation to provide all day UV protection compared to recreational sunscreens. The procedure in this study is modeled after the 2011 FDA regulations for evaluating sunscreens. Several liquid foundations and a sunscreen standard (active ingredients: 7% padimate and 3% oxybenzone) will be tested. UV-vis spectroscopy will be used to perform a broad spectrum test to determine the critical wavelength of each sample. The broad spectrum test will then be used as a base line for comparison to the foundation samples after water exposure at basal temperature. Lastly, the UV-vis spectra of several shades of the same foundation will collected to examine the effect of foundation pigment on UV protection.

**Keywords:**
Consumer Products, Cosmetic, UV-VIS Absorbance/Luminescence

**Application Code:**
General Interest

**Methodology Code:**
UV/VIS
This research seeks to determine the changes that occur in bone during the Alkaline Hydrolysis Cremation (AHC) process. AHC is a form of cremation which is considered by many to be an environmentally friendly alternative to traditional fire cremation. AHC is typically performed under increased pressure and temperature with a basic solution to accelerate the natural decomposition a body typically undergoes. This process results in human or animal remains being completely reduced with only bone ash remaining, which can be returned to the family. The AHC process will be performed using animal bones in a pressure cooker with a basic solution to mimic the commercial process. Bone structure will be characterized before and after the AHC process and the concentrations of calcium, magnesium, and iron metals will be monitored during the AHC process. The bone structure will be analyzed by scanning electron microscopy. Metal analysis will be performed using atomic absorption spectroscopy.

**Keywords:** Atomic Absorption, Biomedical

**Application Code:** General Interest

**Methodology Code:** Atomic Spectroscopy/Elemental Analysis
Many agricultural processes require the application of nitrogen- and phosphate-based fertilizers. Misapplication of these fertilizers can result in the runoff of excess nitrogen and phosphate into local waterways. This pollution has both adverse biological consequences and increases the cost of water treatment. There is a need for rapid, routine and cost-effective quantification of nutrients such as nitrate and phosphate in aqueous systems to monitor runoff and identify sources of pollution. Colorimetric assays for the quantification of nitrate and phosphate are commonly used, but require multi-step sample treatment to be performed. Adapting this analysis for a paper-based microfluidic platform allows for the quantification of nutrient levels on a smaller, less-wasteful scale. Additionally, the implementation of a lateral flow assay enables multi-step reactions to take place on the paper device, allowing for on-site measurements with the use of a smartphone based application. In this way, water quality can be monitored more frequently and the status of the nitrogen and phosphate cycles can be tracked in a more sustainable way.

Keywords: Environmental/Water, Lab-on-a-Chip/Microfluidics, Monitoring, Trace Analysis
Application Code: Environmental
Methodology Code: Microfluidics/Lab-on-a-Chip
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<td>Abstract Title</td>
<td>Synthesis of 2-phenylimidazo [1,2-a]Pyridine: A Development in Medical Chemistry</td>
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<td>Primary Author</td>
<td>Erik W. DiNardo</td>
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<td>Author</td>
<td>California University of Pennsylvania</td>
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<td>Co-Author(s)</td>
<td>Matthew Price</td>
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**Abstract Text**

2-phenyl-1,2imidazopyridines with heteroatoms and amino ortho-substitution on 2-phenyl rings has been reported to display excited state intermolecular proton transfer (ESIPT) with a high stokes shift in a variety of solvents. An efficient route for the synthesis of imidazo[1,2-a]pyridine analogs using 2-aminopyridine derivatives and 2-bromoacetophenones was achieved using a Ortoleva–King reaction is reported. This project will summarize efforts to synthesize imidazopyridine derivatives with varying inductive effects and tuning effects on ESIPT emission wavelengths.

**Keywords:** Biomedical, Derivatization, FTIR, NMR

**Application Code:** Pharmaceutical

**Methodology Code:** Atomic Spectroscopy/Elemental Analysis
Abstract Text

Cytochrome [i][c]/[i] (cyt [i][c]/[i]) is a redox protein found in the mitochondria of all living organisms where it plays an important role in the process of creating cellular energy. Because of its biological importance, as well as its small size, availability, and ease of handling, cyt [i][c]/[i] has been studied extensively. The majority of the studies, both in solution and adsorbed to surfaces, have investigated how the protein’s redox chemistry changes with its environment. Several research groups [sup]1,2[/sup] have studied cyt [i][c]/[i] adsorbed to individual and mixed organothiol self-assembled monolayers (SAM) on gold in pH 7.0 and 4.4 mM phosphate buffers. Excellent voltammetric responses are obtained for cyt [i][c]/[i] adsorbed to the SAM structures under these conditions. The present research probes the redox properties of cyt [i][c]/[i] adsorbed onto gold electrodes coated with short peptide self-assembled monolayers and compares the changes in redox potential and the electron transfer (ET) rates to traditional organothiol carboxylic acid terminated SAMs. Redox properties are determined using cyclic voltammetry. Two different peptides have been designed and synthesized to produce designer electrodes that mimic the redox partners for cyt [i][c]/[i] in the mitochondria. The major changes in the voltammetry for the two new peptide monolayer systems will be presented and explained.

References:


Carbon materials are tremendously important as electrode materials in both fundamental and applied electrochemistry. Recently, significant attention has been given not only to traditional carbon materials, but also to carbon nanomaterials for various electrochemical applications in energy conversion and storage as well as sensing. Importantly, many of these applications require fast electron-transfer (ET) reactions between a carbon surface and a redox-active molecule in solution. It, however, has not been well understood how heterogeneous ET kinetics at a carbon/solution interface is affected by the electronic structure, defect, and contamination of the carbon surface. Problematically, it is highly challenging to measure the intrinsic electrochemical reactivity of a carbon surface, which is readily passivated by adventitious organic contaminants.

In this presentation, we summarize our recent studies of graphite by nanoscale scanning electrochemical microscopy (SECM) not only to reveal the fast ET kinetics of simple ferrocene derivatives at their graphitic surfaces, but also to obtain mechanistic insights into their extraordinary electrochemical reactivity. Specifically, we developed SECM-based nanogap voltammetry to find highly oriented pyrolytic graphite (HOPG) yields an extremely high standard ET rate constant, $k^0$ of $12 \text{ cm/s}$ for (ferrocenylmethyl)trimethylammonium, which was $>10–100$ times higher than reported for air-exposed HOPG by using scanning electrochemical cell microscopy based on the intrinsically contaminating setup. By contrast, we protected the clean surface of HOPG from the airborne contaminants during its exfoliation and handling by forming a water adlayer to obtain the reliable $k^0$ value from symmetric pairs of nanogap voltammograms. This result disproves a recent misconception of simple electrochemical thermodynamics that the non-ideal asymmetry of paired nanogap voltammograms is due to adsorption effects.

**Keywords:** Electrochemistry, Microelectrode, Microscopy, Voltammetry

**Application Code:** Material Science

**Methodology Code:** Electrochemistry
Abstract Text

Scanning ion conductance microscopy (SICM) is a scanned probe microscope in which a nanopipette, containing electrolyte solution and a quasi-reference counter electrode (QRCE), is used to map the topography of interfaces in electrolyte solution without requiring direct mechanical contact with the substrate itself. This makes SICM particularly powerful for the investigation of soft (biological) samples. Traditionally a bias is applied between the QRCE in the nanopipette tip and a second QRCE in bulk solution to generate a direct ion current (DC). Harmonic modulation of the probe position in the direction normal to the surface generates a corresponding alternating ion current which is most often used as a set-point to sense substrate topography. However, analysis of SICM as an electrochemical cell reveals that it could be implemented in new ways that could be advantageous and that it should be particularly powerful for new uses in electrochemistry and beyond. Key advantages of SICM in this context are that: (i) the electrochemical set-up is rather simple and easily understood; (ii) mass transport can be modelled to a high level; (iii) probes can be made at the nanoscale in a matter of seconds and are readily characterized.

In this contribution, I will highlight new directions for SICM and nanopipettes:
(i) New control schemes such as bias modulation that allows much faster scanning
(ii) Charge mapping at functionalized surfaces and living cells
(iii) Reactive imaging at electrodes and modified surfaces
(iv) 2D and 3D patterning, writing and reading
(iv) Integration of SECM and SICM principles to make new probes for surface analysis and imaging
(v) Strategies for fast scanning (functional images in seconds), with approaching 10,000 pixels/second

Further applications of SICM in electrochemistry will be discussed, particularly with regard to pushing electrochemical imaging to new levels.

Keywords: Electrochemistry, Imaging, Surface Analysis
Application Code: Nanotechnology
Methodology Code: New Method
Carbon nanopipettes prepared by chemical vapor deposition (CVD) of carbon into the pre-pulled quartz capillaries can serve as versatile electrochemical nanosensors. Transmission electron microscopy (TEM) was used to characterize these sensors and understand their electrochemical responses. Such sensors can enable detection and quantitation of important analytes (e.g., dopamine) in small spaces. Several nanoprobe geometries presented in this paper include an open carbon pipette, a "nanosampler" with a nanocavity near its tip, and a pressure-controlled pipette containing a variable solution volume. The applications of such pipettes as rectification sensors and collectors for single nanoparticle collision studies are also discussed.
Obtaining molecular-level understanding of cellular communication in biological systems over a range of lengths and time scales is a grand challenge in biology. The complex dynamic nature of biological systems makes it difficult to understand how numerous molecular processes, such as signaling or metabolic pathways are interconnected and controlled within spatial and temporal constraints in living systems. Many imaging techniques have been developed to explore signaling pathways at the subcellular level. However, individual imaging modalities are typically limited to specific molecular properties and biological processes. Simultaneous imaging of chemical gradients produced by multiple biological processes in living cells and tissues with high spatial resolution and exceptional molecular sensitivity and selectivity will provide unprecedented depth of information about molecules involved in communication within and between living cells in their native environment. We have developed a unique platform for imaging live biological systems by coupling nanospray desorption electrospray ionization mass spectrometry imaging (nano-DESI MSI) with electrochemical cell microscopy (ECM). This is achieved using finely pulled precisely positioned small pipettes that facilitate gentle analyte extraction and surface topography mapping. Nano-DESI MSI enables quantitative imaging of numerous metabolites and lipids with a spatial resolution better than 10 [micro]m. Meanwhile, ECM may be used for contact-free mapping of the cell topography, studying protein dynamics at the nanoscale, and quantitative imaging of selected redox-active species in complex biological systems. The design of the system will be presented and its performance for single-cell and tissue imaging will be demonstrated.
**Session Title:** ACS-DAC - Unconventional Pipetting for Bio/Chem Analysis

**Abstract Title:** Imaging via Electrospray

**Primary Author:** Lane A. Baker  
Indiana University

**Co-Author(s):**

**Abstract Text:**
Electrospray from nanopipettes is used to realize scanning electrospray microscopy (SESM). This technique provides an ambient, non-contact method to investigate surface topography with distance-dependence of electrospray current as feedback for imaging. SESM approach curves, line scans, and images are reported. Salt deposition on the sample surface from SESM is characterized.

**Keywords:** Electrode Surfaces, Electrospray, Imaging, Instrumentation  
**Application Code:** Bioanalytical  
**Methodology Code:** Mass Spectrometry
Great advances have been made in analytical instrumentation for industries including Environmental, Botanical Dietary Supplements, Food Safety and Clinical Chemistry. The emerging cannabis testing and research markets have benefited tremendously from advances in these industries. To date, most of the analytical cannabis applications have centered on quality control. This presentation will focus on efforts to bridge the gap between these analytical techniques and medical cannabis applications, in order to bring together experts from both arenas to share knowledge and improve cannabis testing.

Most analytical experts in industry and academia have extremely limited or no access to cannabis samples. Likewise, as more and more states are moving towards medicinal cannabis programs, doctors have very little training in cannabis and the endocannabinoid system. Clinical chemistry / toxicology labs may be familiar with THC testing for drugs of abuse and pain management applications, but lack the testing methodologies necessary for cannabinoid therapeutic drug monitoring or personalized medicine strategies. This talk with showcase recent successes in bringing medical, analytical and cannabis experts together to develop a new era of cannabis science. This presentation will also demonstrate new research opportunities to use analytical solutions to begin to unlock the mysteries of medical cannabis therapies. One example is the application of functional Near Infra-red Spectroscopy, or fNIRS, to monitor brain activity in real-time, giving us the ability to study the effects of cannabis administration on brain function.

Keywords: Clinical/Toxicology, Near Infrared, Quality Control, Spectroscopy
Application Code: Bioanalytical
Methodology Code: Near Infrared
Many medical researchers have widely criticized the Federal Government's stance to keep Cannabis on Schedule 1, effectively keeping cannabis out of the hands of researchers. While cannabis is only one plant, there are hundreds of strains, comprised of many different cannabinoids and terpenes, which are the chemical compounds secreted by cannabis flowers. When cannabis is consumed, the cannabinoids bind to receptors sites throughout the brain and body. Different cannabinoids and compounds have different therapeutic affects based on which receptors they bind to; this proven theory is the cornerstone of all medical research with relations to cannabis also coined as the “Entourage Effect” introduced by S. Ben-Shabat and Dr. Raphael Mechoulam in 1998.

Due to the complex structure of the different cannabinoids and terpenes of and varying strains of cannabis, researchers are only now just scratching the surface of determining this plant capabilities for medical uses. One thing is certain that when cannabis is grown in a clean, consistent, and stabilized environment, it is optimal for scientific research. We will talk about the optimal environmental parameters in which cannabis is grown and how these indoor grow facilities are constructed with the overall goal to minimize the introduction, generation, and retention of airborne particulates, and pest and pathogens within the facility. While maintaining control of all other relevant parameters such as temperature, humidity, and overall room pressure. Materials we use in indoor cultivation techniques are similar to materials used in labs, clean rooms, and commercial kitchens. In addition, we will cover HVAC bio contamination risks and the current climate of medical research.

**Keywords:** Agricultural, Air, Automation, Natural Products

**Application Code:** Agriculture

**Methodology Code:** Education/Teaching
With new regulatory requirements for Cannabis testing in Oregon to be implemented in 2016, analytical testing laboratories are faced with the challenge of preparing uniform, representative samples for chemical analysis that are relevant for a 4.5 kg (10 lb) production batch. Current sample preparation methods that may include coffee grinders or kitchen blenders, may not provide the speed & reproducibility that is required for the analytical method. Moreover, given the variability of cannabinoids, terpenes, and potential chemical or microbial contaminants that may occur within a given crop, or even within the same plant, the random selection of certain flowers may not provide analytical data that is truly reflective of the entire crop.

The production of pre-rolled smokable products, and certain methods for oil extraction, typically involve a milling or grinding procedure to obtain an optimal particle size & consistency. It is proposed that the milling & homogenization of the entire Cannabis batch prior to collecting sample material for analysis, may provide for a more uniform & representative chemical analysis. The data presented shows the variability between stated potency, re-analysis of dried flowers, and samples obtained post-milling.

Keywords: GC-MS, HPLC, Method Development, Sampling
Application Code: Process Analytical Chemistry
Methodology Code: Sampling and Sample Preparation
The legal cannabis industry is exploding as more states adopt medical and recreational use laws. Cannabis is still federally illegal so the legal cannabis market is managed by local state governments. While systems for growing, production and sale of cannabis and cannabis related products are well established, regulation and enforcement of quality and safety testing have lagged behind. But now state governments and private labs are focusing on product safety testing with special emphasis on pesticide analysis. This is the result of various product recalls, media attention and concern from patient advocacy groups.

We developed a modified QuEChERS LC-MS/MS method for analysis of multiresidue pesticides. The AOAC QuEChERS method was used for a reduced 1.5 g amount of plant material and processed with Universal dSPE. LC-MS/MS analysis used constant polarity switching ESI and monitored at least two transitions per analyte. Matrix-matched calibration was used for quantitation and both method and instrument internal standards were used. Analyte recovery validation was performed according to FDA guidelines by testing three matrices at three fortification levels in triplicate for over 200 pesticides. For the large majority of pesticides, in all three matrices and at all three fortification levels, recovery was between 70-120%.
Analytical Cannabis I

The Analytical Potential of a Compact Mass Spectrometer (CMS) for the Analysis of Cannabis-Related Samples for Composition and Adulteration

The current rapidly growing aspects of the cannabis industry raises many medical, legal, social and analytical questions. Due to the often chemical complexity of representative samples including the presence of isobaric analogs chromatography coupled with sensitive and selective detectors are often needed. In contrast, in some instances questions needing quick answers may be obtained by simple, modern analytical technologies without the benefits of chromatographic separation or high resolution mass spectrometry. Thus screening techniques by a simple mass spectrometer can often identify which samples need further study and which ones do not.

Although high performance triple quadrupoles, QTOF’s and orbitraps are often selected for these applications, this presentation will describe how often some questions can be quickly answered by simpler and cheaper approaches. In this presentation we will describe how the determination of cannabis-related compounds may be determined utilizing a novel compact mass spectrometer (expression-S, Advion, Inc. NY) coupled to ultra-high performance liquid chromatography (UHPLC) or even thin layer chromatography (TLC).

The described compact, transportable single quadrupole mass spectrometer may be coupled with sophisticated on-line UHPLC sample analysis (UHPLC/MS) or with simple TLC plates (TLC/MS) following a preliminary sample cleanup step. In addition, a variety of simple ambient ionization techniques including atmospheric solids analysis probe (ASAP) and the open port source ionization (OPSI) inlet system recently reported by ORNL may be employed. These latter techniques often require no prior sample preparation or chromatographic separations.

Examples will range from the analytical determination of the quality and purity of cannabinoid products as well as the presence of pesticides and economic adulterants that may be present. The samples will range from unknown products and powders to plant materials.

Keywords: Forensic Chemistry, Liquid Chromatography/Mass Spectroscopy
Application Code: Biomedical
Methodology Code: Mass Spectrometry
Analytical Techniques for Probing Neurochemistry

Microdialysis-Microchip Electrophoresis for Continuous Monitoring of Neuroactive Substances in the Brain

The goal of this work is to develop a miniaturized device that can be employed for the continuous monitoring of drugs and neurotransmitters in the extracellular fluid of the brain. Two applications are envisioned. The first is on-animal sensing so that neurochemistry can be correlated with behaviour. The second is for use in a hospital setting to evaluate patients with traumatic brain injury. This device combines microdialysis sampling with microchip electrophoresis. Microdialysis (MD) sampling provides relatively clean protein-free samples with no net fluid loss in the brain. Microchip electrophoresis (ME) makes it possible to separate and detect several analytes in a single run with good temporal resolution. Systems using both electrochemical and fluorescence detection are being developed for neurotransmitters and amino acids, respectively. Progress on the development of MD-ME systems for catecholamines, adenosine, and amino acid analysis will be reported.

Keywords: Biomedical, Electrochemistry, Lab-on-a-Chip/Microfluidics, Neurochemistry
Application Code: Neurochemistry
Methodology Code: Microfluidics/Lab-on-a-Chip
Analytical Techniques for Probing Neurochemistry

Neurochemistry in the Intensive Therapy Unit – Faster, On-Line Multi-Analyte Analysis for Traumatic Brain Injury Patients

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. New analytical instrumentation has a vital role in devising new treatment strategies.


Keywords: Biosensors, Electrochemistry, Ion Selective Electrodes, Lab-on-a-Chip/Microfluidics

Application Code: Neurochemistry

Methodology Code: Microfluidics/Lab-on-a-Chip
Microdialysis is a popular and powerful method for sampling the extracellular space of the brain and other neural tissues. A challenge arises in microdialysis procedures because inserting the probe into the tissue causes an unavoidable traumatic penetration injury. Due to the penetration injury, the tissue near the probe is not normal and behaves in a manner quite different from healthy, non-traumatized tissue. Also, the traumatized tissue launches an inflammatory response to the trauma, which is the mechanism of recovery from the injury. This wound healing response of the host tissue has two important consequences. First, the tissue changes over time following the initial implantation of the probe. Second, eventually the wound healing response produces scar tissue that prevents further collection of meaningful data. By the incorporation of an anti-inflammatory agent, dexamethasone, into the microdialysis perfusion fluid, we show that the trauma is minimized, that the wound healing response is dampened, and probes provide meaningful data for unto 10 days following implantation (the longest time yet attempted). We believe that the relatively simple step of adding an anti-inflammatory agent to the microdialysis perfusion fluid represents a vast expansion of the capabilities and opportunities for intra-cranial chemical monitoring.
Depression is a devastating mental disorder that the World Health Organization predicts will be a leading disease burden by 2030. Unfortunately, the precise neurochemistry underlying depression is not well understood, which makes pharmaceutical therapies have variable benefits and low efficacy rates. Additionally, many drug companies have toned down their efforts to develop novel drugs since precise pharmaceutical targets are unknown. In this work, we employ fast-scan cyclic voltammetry (FSCV), fast-scan controlled-adsorption voltammetry (FSCAV), mouse behavior and mathematical modeling to elucidate the fundamental differences in serotonin between healthy and behaviorally-depressed mice. Male and female mice are behaviorally-stressed using a chronic unpredictable mild stress protocol, and the level of their depression is interrogated with a battery of behavioral tests. FSCV and FSCAV are then applied in the mouse hippocampus to make real-time, quantitative evoked and ambient serotonin measurements. Finally, experimental data is mathematically modeled to determine mechanistic differences in the regulatory mechanisms controlling extracellular serotonin in healthy and depressed mice. The insight gained from this research, revealing important differences in the serotonin system in a depression model, has the potential to better focus therapeutic efforts.

Keywords: Bioanalytical, Electrochemistry, Neurochemistry, Voltammetry
Application Code: Neurochemistry
Methodology Code: Electrochemistry
Analytical Techniques for Probing Neurochemistry

Prefrontal Orbital Network Dynamics in Chronic Stress and Hyperexcitable States

Medial prefrontal cortex (mPFC) dysfunction has emerged as a consistent locus of pathology in depression and other stress-related psychiatric conditions, as well as an important therapeutic target for deep brain stimulation. Convergent evidence from chronic stress models in rodents and from spectroscopy and postmortem studies in patients suggest that hyperexcitability, elevated metabolic activity, and synapse loss in the mPFC may contribute to anhedonia and other depression-related behaviors, but the underlying mechanisms remain poorly understood. Whether and how changes in the excitability of mPFC pyramidal cells affect synaptic remodeling and mPFC microcircuit function is unknown. To address these questions, we conducted two sets of studies. First, we used stable step function opsins and a chronic stress model to manipulate the excitability of mPFC pyramidal cells and test for effects on the remodeling of postsynaptic dendritic spines, assessed by repeated two-photon imaging through chronically implanted microprisms in Thy1/YFP transgenic mice. Second, we used two-photon calcium imaging to test for associated changes in mPFC network dynamics. We found that postsynaptic spine remodeling was accelerated in hyperexcitable and chronic stress states, leading to an accumulating loss of synapses over time. Gradually accumulating synapse loss was associated with widespread changes in functional connectivity and in graph metrics of network efficiency and clustering that were especially pronounced in highly connected, “hub-like” neurons and were correlated with increased anhedonia- and anxiety-like behavior. We are currently testing whether optogenetic and pharmacological interventions to reverse stress-induced changes in mPFC excitability are sufficient to rescue dysfunctional network dynamics and behavior.

Keywords: Neural Network, Neurochemistry
Application Code: Neurochemistry
Methodology Code: Microscopy
Effective diagnostic tests for tuberculosis (TB) and many other infectious diseases are critical for improved patient care and global infection control. TB is one of the world’s deadliest infectious diseases, alongside HIV/AIDS. This presentation describes the development and preliminary testing of a heterogeneous immunoassay for TB that is based on the low-level detection of lipoarabinomannan (LAM). LAM, a lipoglycan, is a major virulence factor in the infectious pathology of TB and has been found in the serum, sputum, and urine of infected patients. The first part of the presentation details the development of an assay platform for the determination of LAM, based on gold nanoparticle labels, monoclonal antibodies, and surface-enhanced Raman scattering (SERS). We have found that this detection platform, when applied in combination with sample pretreatment, enables the effective measurement of LAM in TB-infected patient sera. The second part of the presentation examines the potential to detect TB at point-of-need (PON) and focuses primarily on the effectiveness of measuring SERS responses with a small, handheld Raman spectrometer. Together, this work not only provides important evidence for the clinical utility of LAM as a TB biomarker, but also demonstrates the potential to deploy this test in TB-endemic regions of the world which are often resource limited. Prospects and challenges to the extension of this approach for use in clinics and PON settings, along with possible applications to other TB markers and types of patient specimens, are examined and discussed.

Keywords: Bioanalytical, Biosensors, Portable Instruments, Surface Enhanced Raman Spectroscopy
Application Code: Bioanalytical
Methodology Code: Portable Instruments
Evolving Spectroscopic Technologies for Point-of-Origin Detection of Diseases and Environmental Toxins

Metabolic Profiling by SERS: A Diagnostic for Bacterial Infections

Surface enhanced Raman spectroscopy (SERS) spectra excited at 785 nm on Au and Ag nanostructured substrates provide sensitive and specific vibrational signatures for bacterial diagnostics, cancer cell identification, and measures of blood aging. A SERS based methodology for rapid, growth-free diagnostics of blood infections, urinary tract infections (UTIs), and sexually transmitted diseases (STDs) has been developed. The acquisition of SERS spectra of the bacteria enriched from infected body fluids, combined with multivariate data analysis techniques and a drug-susceptibility determined SERS library, results in rapid, antibiotic specific bacterial diagnostics. The dominant molecular species contributing to the 785 nm excited SERS spectra of bacteria are the metabolites of purine degradation: adenine, hypoxanthine, xanthine, guanine, uric acid and AMP. These molecules result from the starvation response of the bacterial cells in pure water washes following enrichment from nutrient rich environments. Vibrational shifts due to isotopic labeling, bacterial SERS spectral fitting, SERS and mass spectrometry analysis of bacterial supernatant, SERS spectra of defined bacterial mutants, and the enzymatic substrate dependence of SERS spectra are used to identify these molecular components. The absence or presence of different degradation/salvage enzymes in the known purine metabolism pathways of these organisms plays a central role in determining the bacterial specificity of these purine-base SERS signatures. These results provide the biochemical basis for the development of SERS as a rapid bacterial diagnostic and illustrate how SERS can be applied more generally for metabolic profiling as a probe of cellular activity.

Abstract Text

Surface enhanced Raman spectroscopy (SERS) spectra excited at 785 nm on Au and Ag nanostructured substrates provide sensitive and specific vibrational signatures for bacterial diagnostics, cancer cell identification, and measures of blood aging. A SERS based methodology for rapid, growth-free diagnostics of blood infections, urinary tract infections (UTIs), and sexually transmitted diseases (STDs) has been developed. The acquisition of SERS spectra of the bacteria enriched from infected body fluids, combined with multivariate data analysis techniques and a drug-susceptibility determined SERS library, results in rapid, antibiotic specific bacterial diagnostics. The dominant molecular species contributing to the 785 nm excited SERS spectra of bacteria are the metabolites of purine degradation: adenine, hypoxanthine, xanthine, guanine, uric acid and AMP. These molecules result from the starvation response of the bacterial cells in pure water washes following enrichment from nutrient rich environments. Vibrational shifts due to isotopic labeling, bacterial SERS spectral fitting, SERS and mass spectrometry analysis of bacterial supernatant, SERS spectra of defined bacterial mutants, and the enzymatic substrate dependence of SERS spectra are used to identify these molecular components. The absence or presence of different degradation/salvage enzymes in the known purine metabolism pathways of these organisms plays a central role in determining the bacterial specificity of these purine-base SERS signatures. These results provide the biochemical basis for the development of SERS as a rapid bacterial diagnostic and illustrate how SERS can be applied more generally for metabolic profiling as a probe of cellular activity.

Keywords: Bioanalytical, Biomedical, Surface Enhanced Raman Spectroscopy, Vibrational Spectroscopy

Application Code: Biomedical

Methodology Code: Vibrational Spectroscopy
We report a handheld LIBS spectrometer for measuring mineral deficiency in human tissue. The LIBS spectrometer is based on a high repetition rate pulsed laser. The laser generates a train of laser pulses at a high repetition rate (in the kHz range), which produces several thousands of micro-plasma emissions per second from the tissue. A synchronized miniature CCD array spectrometer integrates the LIBS signal produced by this plurality of laser pulses. Hence the intensity of the obtained LIBS spectrum can be greatly improved to increase the signal-to-noise ratio (SNR) and lower the level of detection (LOD). As one example, we measured the LIBS spectra of nail and hair samples from multiple individuals. The nail measurement was performed directly on the individual’s finger. The hair measurement was performed on a lock of hair freshly cut from the same person. The following figure shows a comparison of the collected LIBS spectra from two individuals, where Figures (a) and (a) are the spectra of the nail and hair sample from individual #1, and Figures (b) and (b) are the spectra of the nail and hair sample from individual #2. It is interesting to note that the trace element concentration in nail and hair is consistent for the same individual. For example, individual #1 has higher Cu concentration in both her nail and hair, while individual #2 has higher Zn concentration in both her nail and hair. This verifies the capability of the LIBS spectrometer for performing on-site mineral deficiency test in human bodies.
Development of diagnostic methods for rapid and sensitive identification of biomedical pathogens is essential for the advancement of therapeutic and intervention strategies necessary to protect public health. Current diagnostic methods, e.g. culture, isolation, PCR, antigen detection, and serology, are often time-consuming, cumbersome, or lack sensitivity. We have investigated several different nanoparticle platforms for surface-enhanced Raman (SERS)-based identification and classification of pathogens. These platforms included metal colloids, nanosphere arrays, OAD nanorod arrays, and layer-by-layer nanoparticle assembly. The current talk will address the development of spectroscopic methods for pathogen detection based on these nanostructured SERS platforms. This presentation will describe the use of these nanofabricated arrays in conjunction with SERS for direct detection of the respiratory pathogen Mycoplasma pneumoniae. An overview of the challenges and successes that have marked progress toward a real-time SERS-based diagnostic platform for these bacteria is described, as well as strategies employed to address future clinical applications.
Evolving Spectroscopic Technologies for Point-of-Origin Detection of Diseases and Environmental Toxins

Structure and Morphology of Biosynthesized and Biodegradable Polymer Nanofibers, Ultrathin Films and Single Crystals Using AFM-IR and Selected Area Electron Diffraction (SAED)

Nanofibers, ultrathin films and isothermally grown single crystals of poly[(R)-3-hydroxybutyrate-co-(R)-3-hydroxyhexanoate] (PHBHx) have been produced with a wide range of crystallinity and molecular orientation. They have been characterized using polarized AFM-IR spectroscopy, IR imaging and SAED 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 ultrafine electrospun fibers at the single fiber scale and single crystals as thin as 13 nm. 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.

The homopolymer, poly(hydroxybutyrate) (PHB) is a biodegradable, aliphatic polyester that can be produced by chemical processes or bacterial fermentation. The fact that it can be produced biologically with the properties of a thermoplastic and is naturally biodegradable, have made it a subject of research in many industrial and academic laboratories worldwide. However, bacterially produced PHB results in a 100% stereoregular, highly crystalline polymer that is brittle and lacks flexibility. In order to modify these properties 3-hydroxyhexanoate (3HHx) has been added as a co-monomer and these new materials, referred to as PHBHx, exhibit a significantly reduced crystalline content, resulting in improved mechanical properties and processability.


Keywords: Biosensors, Environmental/Water, Molecular Spectroscopy, Raman Spectroscopy
Application Code: Environmental
Methodology Code: Sensors
Secreted factors play an important role in normal and pathological processes in every tissue in the body. The brain is composed of different cell types and few methods exist that can identify which individual cells in a complex mixture are secreting specific analytes. Multiple neurodegenerative diseases are characterized by the abnormal accumulation of toxic proteins that lead to cell death. In Alzheimer’s disease, this protein is called Amyloid beta (A[beta]). Here, we describe a methodology for detecting A[beta] at a single cell level. By identifying which cells are responsible, we can better understand neural physiology and pathophysiology, more readily identify the underlying pathways responsible for analyte production, and ultimately use this information to guide the development of novel therapeutic strategies that target the cell types of relevance. To detect A[beta] secretion from single cells, we optimized a method called microengraving, which we coupled to immunostaining and single cell RNA sequencing. We apply this methodology to study A[beta] secretion from multiple cell types including human astrocytes and stem cell derived neurons. Through these studies, we have uncovered the dynamic range of secretion profiles of these analytes from single iPSC-derived neuronal and glial cells and have molecularly characterized subpopulations of these cells through immunostaining and gene expression analyses. By examining single cell secretion of A[beta] and related analytes, we have identified previously unappreciated complexities in the biology of production of these species that could not otherwise have been found by studying averaged responses over pools of cells. This technique can be readily adapted to the detection of other analytes secreted by neural cells, which would have the potential to open new perspectives into human CNS development and dysfunction.

**Keywords:** Bioinformatics, Biomedical, Lab-on-a-Chip/Microfluidics, Single Molecule

**Application Code:** Biomedical

**Methodology Code:** New Method
Methods for spatiotemporal tracking of cells are becoming increasingly important, especially as interest in cell-based therapies continues to grow. Such methods can shed light on migration routes and biodistribution of cells, which may be important markers of treatment efficacy. In addition, cell tracking methods are key for analyzing the migration patterns of various types of circulating cells, including immune cells, cancer cells, and stem cells. However, current methods for in vivo cell tracking are not sufficiently sensitive to be applied to tracking single cells at the whole-body level. It has been hypothesized that single cells scattered throughout a tissue may produce a significant therapeutic effect. Furthermore, single cancer cells possess the ability to form entire metastases. We have developed a novel method for extracting the position of single cells from raw PET measurements. Further, we have successfully demonstrated that we can track single cell-mimicking droplets containing 100-200 Bq of radioactivity. This level of radioactivity is however still higher than what is achievable with current cell radiolabeling protocols. Labeling with 18F-fluorodeoxyglucose (18F-FDG) yields approximately 5-10 Bq per cell. We are therefore aiming to close this gap with a multipronged approach. We are investigating the use of a novel PET system made from bismuth germanate detectors, which have exceedingly low background, suitable for tracking few cells. We are also developing algorithms more robust to scattered and background radiation. In parallel, we are optimizing the radio labeling of cells using a radiotracer known as 18F-HFB. This radiotracer has previously been shown to label cells more efficiently that 18F-FDG, reaching up to 25 Bq per cell. Combining these approaches should allow us to close the gap between what is needed and what can be achieved, and enable the tracking of single cells in vivo, anywhere in the body.
A tumor consists of highly heterogeneous cell populations with a majority of normal tumor cells and rare subsets of cancer stem cells (CSCs). A few CSCs in a tumor can cause cancer to progress, recur and spread, because they can self-renew, differentiate and resist treatment. Therefore, it is crucial to identify and study rare subsets of CSCs in the tumors at single-cell resolution in order to determine their roles in cancer aggression, invasion and metastasis. A single CSC may only express a very few distinctive receptor molecules. Thus, single molecule sensitivity and multiplexing capability are needed to quantify these biomarker receptors on individual cells in order to effectively identify rare subsets of CSCs among tumor cells. Current methods (flow cytometry, fluorescence-based assays) cannot offer sufficient selectivity, sensitivity, temporal and spatial resolutions to quantitatively detect individual receptor molecules on single CSCs and understand their functions in situ. Rare subsets of single CSCs have to be isolated or pre-concentrated for further analysis. Thus, single CSCs are not studied among other tumor cells in their native environments over time, which hinders the study of their roles in tumor growth, invasion and metastasis, and design of more effective diagnosis and therapy.

We have developed a novel molecular imaging platform, including photostable multicolored and multifunctional single molecule nanoparticle optical biosensors (SMNOBS) and far-field photostable optical nanoscopy (PHOTON) for real-time study of functions of single live cells. We have demonstrated that we can use these new nano tools to quantitatively image and characterize roles and functions of multiple individual receptor molecules on rare subsets of single CSCs in highly heterogeneous tumor populations for earlier cancer detection. The detailed experimental design and updated results will be discussed.

Keywords: Bioanalytical, Imaging, Nanotechnology, Single Molecule
Application Code: Nanotechnology
Methodology Code: Sensors
Over 50% of cancer patients harbor a mutation in the tumor suppressor protein p53. In healthy cells during stress, p53 recognizes specific genomic response elements (REs) to induce expression of stress response genes involved in cell cycle arrest, apoptosis and DNA damage. To maintain genomic stability, expression of these tumor suppression genes must be rapidly turned on and off. It is currently poorly understood how p53 spatiotemporally regulates expression of target genes in the complex milieu of the nucleus and how this process goes awry in cancer cells.

To better mechanistically decipher changes in stress response gene expression in cancer cells, we established a single molecule imaging system that allows us simultaneously to track p53 and RNA Polymerase II binding along with transcription dynamics in single live cells. Single particle tracking studies indicate a dynamic interaction between p53 and chromatin that varies in distinct sub-nuclear compartments. Live cell monitoring of gene expression from a p53 target gene reveals dynamic bursts of transcription in discrete sub-nuclear regions. Modulation of wild-type p53 levels leads to changes in the transcription burst frequency and intensity. Introduction of cancer associated mutant p53 into cells leads to changes in both p53 sub-nuclear localization and transcription bursting dynamics. Future studies will focus on how chromatin structure dynamically regulates sub-nuclear localization and expression of p53 targets genes. This work is supported in parts via the 4D Nucleome consortia NIH grant 1U01EB021236-01.
Abstract Text
We have recently demonstrated a technology using sequential hybridization and single molecule FISH to multiplex a large number of mRNA molecules directly in single cells in complex tissue samples. mRNAs in cells are barcoded by sequential rounds of hybridization, imaging, and probe stripping. The number of barcodes available with this approach scales as \( F^N \), where \( F \) is the number of distinct fluorophores and \( N \) is the number of hybridization rounds. We call this method seqFISH and it is conceptually akin to “sequencing” mRNAs directly in cells by FISH. We will discuss application of this technology to brain sections, embryos, and human tissues.
Mass spectrometry is being applied to solve problems of urgent health crises, including an aging population that is steadily increasing in size. Our group has been interested in the intersection of aging and immunity to better understand host response to infections and ways to improve health status. Using C. elegans as a model system we have begun to follow age-related proteome-wide changes in response to human opportunistic pathogens such as Pseudomonas aeruginosa. The studies involve mass spectrometry-based proteomics to measure proteins from young and middle-aged adult worms using isotopic labeling and isobaric tagging methods. This presentation will provide an overview of mass spectrometry applications to aging and include our findings in C. elegans that demonstrate that there is a significant influence on host response due to aging. Additionally, the implications of our model studies for relevance to human aging will be discussed.

Keywords: Bioanalytical, Mass Spectrometry, Proteomics

Application Code: Bioanalytical

Methodology Code: Mass Spectrometry
Mankind has long recognized the need for measurement science and standards to support construction, manufacturing and trade. During the past 100+ years, National Metrology Institutes – like NIST have developed sound programs for physical metrology focused on the realization of SI units for time, mass, length, temperature, electricity, etc.

But modern society also requires confidence in the comparability of measurements regarding the composition, structure and properties of diverse types of “stuff” to underpin sound decisions about things that affect the quality of our everyday lives. Decisions based on chemical measurements affect the safety and quality of our food, air and drinking water; our health status and the most efficacious treatments on an individual basis; functional properties, performance and reliability of materials needed in a wide range of sectors such as transportation, housing, manufacturing; our personal identity and guilt and innocence of various crimes.

This talk will focus on the need, growth and impact of programs focused on metrology in chemistry, biology and materials science within organizations like NIST, other National Metrology Institutes around the world, and society in general.

Keywords: Bioanalytical, Clinical Chemistry, Food Safety, Materials Characterization
Application Code: Quality/QA/QC
Methodology Code: Chemical Methods
Recognizing Cutting-Edge Chemistry from the National Organization for the Professional Advancement

The Path Toward Urine Albumin Standardization

Urinary excretion of albumin is a major diagnostic and prognostic marker of renal dysfunction and cardiovascular disease; therefore, accurate measurement of urine albumin is vital to clinical diagnosis. Although assay differences and analyte heterogeneity have been reported for urine albumin measurements, accuracy assessments of the available methods have been hindered by the lack of a reference measurement system, including reference measurement procedures and reference materials, for this clinical analyte. The development of a urine albumin reference measurement system will create a chain of traceability that will link routine clinical measurements to SI units. To address urine albumin measurement imprecision, we have developed the following components of the urine albumin reference measurement system: a multiplexed candidate reference measurement procedure that utilizes isotope dilution-mass spectrometry (ID-MS) and multiple reaction monitoring (MRM) to quantify full-length urine albumin; a primary reference material to be used as a calibrator for higher-order urine albumin methods; and a secondary reference material to be used as a quality assessment tool for clinical laboratories or assay manufacturers to verify the accuracy of their urine albumin measurements.

Keywords: Bioanalytical, Clinical Chemistry, Proteomics, Quantitative
Application Code: Biomedical
Methodology Code: Liquid Chromatography/Mass Spectrometry
Analytical Chemist plays a prominent role in problem solving in Research & Development organizations. My talk will focus on highlighting the different roles of the industrial Analytical Chemist at P&G. A discussion on the job market, education requirements and real problems solved at P&G will be discussed during this talk. Students will learn about the “framework” for approaching and solving problems by Analytical Chemist. Additionally, I will share personal highlights involving driving analytical solutions (Chromatography & Polymer Material Characterization) across three major business units in my career at P&G.
A new, ultra-high field, 21T FTICRMS system has been developed at EMSL/PNNL and is now available to the scientific user community for analysis and characterization of complex organic and biological samples that contain a myriad of molecular constituents. The system offers extremely high mass resolution (>3M m/Δm) and mass accuracy (<0.1 ppm). This paper will describe the system and its use for characterization of natural organic matter (e.g. soil organics) and biological samples (e.g. proteomes). A particular application case study will be highlighted where the approach is used to identify and discover Fe-containing compounds (siderophores) that are secreted by microbial systems to improve the bioavailability of this important nutrient. Using this approach the high mass resolution enables elucidation of molecular formula and structure while also providing conclusive confirmation of Fe incorporation through observation of individual Fe isotopes in the molecular mass spectra. The system thus has unique applicability to the characterization of metallomic samples.
We are using laser ablation (LA)-ICP-MS to image the local distribution of elements (metals and hetero-elements) directly or (metallo-)proteins by metal-tagged antibodies in cells and tissue indirectly. Different applications will be discussed to demonstrate the state of the art and to visualize the elemental distribution pattern in soft bio-materials (tissue, single cells).

In the first application Pt-containing drugs for cancer treatment are investigated and elemental distribution pattern are shown for tissue samples from animal experiments. Different standardization and quantification schemes including isotope dilution analysis will be discussed.

In the second application, which is dedicated to toxicological research, the up-take of nano-particles by single cells are discussed and metal containing stains are used to visualize the distribution of nano-particles, proteins and DNA in a single cell simultaneously. This information is correlated with the distribution of the nanoparticles to identify the cell compartments where nano-particles are enriched. Quantification schemes have been developed to transform the measured intensities into number of particles up taken by the cells.

In the third and last application LA-ICP-MS is applied to visualize the local distribution of proteins, which are used as biomarkers for prostate cancer. For this purpose, biopsy samples from patients have been simultaneously stained by eight differently metal-tagged antibodies in a multiplex approach. Detection of house-keeping proteins serves as internal standards to overcome differences in protein expression. Additionally ink-jet printing of metal doped inks onto the surface of these tissue samples has been applied for internal standardization and drift corrections.

Finally future trends to develop an “elemental microscope” will be discussed.

Keywords: Bioanalytical, Elemental Analysis, ICP-MS, Laser
Application Code: Clinical/Toxicology
Methodology Code: Mass Spectrometry
Bio-LIBS and the Role of Trace Metals When Laser-Induced Breakdown Spectroscopy is Used to Study Biological or Biomedical Systems

In recent years, it has been shown that the atomic emission spectroscopy technique of laser-induced breakdown spectroscopy (LIBS) can provide a sensitive elemental assay of both prokaryotic and eukaryotic cells. While these cells are primarily composed of water (hydrogen, oxygen) and carbon, the dominant atomic species that are observed in the plasma emission from ablated cells are the trace metals that are present in the cell such as calcium, magnesium, sodium, and potassium. On the basis of this assay, numerous biomedical diagnostic tests have been proposed. Our group has demonstrated that sensitive and specific discrimination between bacterial specimens at both the species and strain levels is possible and that assays of in vivo human fingernails for a determination of human nutriture are feasible.

In this talk I will review the methodology that has been used to obtain high signal-to-noise LIBS spectra (e.g. from human fingernails, Fig. A) and summarize the applications of the technique in the field of biomedical diagnostics. I will present our group’s recent work to understand the impact of the elemental content of the growth environment on the LIBS spectrum obtained from bacterial cells with a focus on experiments to intentionally dope E. coli cells with zinc and magnesium. Cells showed a controlled zinc uptake proportional to the environmental concentration (Fig. B) and a well behaved concentration curve was obtained (Fig. C). Magnesium lines are generally among the largest observed in bacterial spectra, but as reproducing E. coli cells were exposed to progressively higher concentrations of environmental Mg, the intensity of the Mg LIBS emission lines was largely unchanged while the deviation in measured intensity was reduced (Fig. D).

These studies are relevant for LIBS measurements on cells obtained from medical specimens for infection or health diagnosis as well as on cells obtained from an environmental setting for ecological surveillance.

Keywords: Atomic Emission Spectroscopy, Biomedical, Environmental/Biological Samples, Trace Analysis
Application Code: Biomedical
Methodology Code: Atomic Spectroscopy/Elemental Analysis
Mass Cytometry has enabled massively multi-parameter analysis of single cells at high throughput. A sample of whole cells is probed with a panel of antibodies distinctively tagged with metal isotopes that serve to code their corresponding functional and cell differentiation markers. The tag signature of each cell is registered by a fast ICP-Time of Flight mass spectrometer. The method has extended the capability of flow cytometry of cell suspensions, exposing the deep heterogeneity of biology at the single cell level.

The author believes that another transformation in bioanalytical capability extends the application of the multi-dimensional capabilities of Mass Cytometry to spatial imaging of tissues at sub-cellular resolution. In Imaging Mass Cytometry (IMC), a thin tissue sample on a microscope slide is probed with a panel of isotope-tagged antibodies. The sample is subjected to high speed laser ablation using a quintupled YAG laser having a spot size of a micron or less. A new ablation cell delivers the plume resulting from each individual laser pulse to the ICP-MS instrument such that each pixel is resolved from its neighbor even at greater than 100 Hz. The result is a biomarker and functional protein map of the tissue at cellular resolution. Examples of the application of IMC will be presented with data from collaborators at ETH (Detlef Gunther), U Zurich (Bernd Bodenmiller), Princess Margaret Hospital (David Hedley), and Fluidigm Canada (the author’s former technology development partners).

We will focus on recent work from PMH and Fluidigm where platinum localization was visualized in tissue sections from tumor and normal tissues of cisplatin-treated mice bearing pancreas cancer patient-derived xenografts. In particular, a striking and unanticipated finding was the extensive binding of Pt to collagen fibres, and the subsequent slow release of stroma-bound Pt. Accumulation of Pt in renal tubules is consistent with the nephrotoxicity of cisplatin.

Keywords: Atomic Spectroscopy, Bioanalytical, Biotechnology, Imaging
Application Code: Bioanalytical
Methodology Code: Atomic Spectroscopy/Elemental Analysis
The field of metallomics presents some of the greatest challenges for analytical science. Not only must a broad suite of chemical elements be determined, the concentration range over which they might be present extends over as many as 14 orders of magnitude. In addition, it is not only the chemical elements that must be determined, it is necessary also to identify and quantify the compounds, ions, and biochemical entities with which they are associated. As an added complication, the associations are often relatively unstable, so the analysis must be “tunable” in how much energy it imparts to the species being determined.

In this presentation, the adaptation of a relatively new atomization/ionization source to metallomic analysis will be described and evaluated. Termed the Solution-Cathode Glow Discharge (SCGD), the source has already been applied successfully to the determination of elemental concentrations by atomic-emission spectrometry. However, here the SCGD will employ mass spectrometry for detection, and will target not only chemical elements, but also adducts and other species with which the elements are associated. Moreover, mass spectra produced by the source can be “tuned” to indicate alternatively atomic ions, associated entities, or intact molecules. Further, it will be shown that SCGD-MS can be used to identify polypeptides and even hint at their amino-acid sequences.

Keywords: Bioanalytical, Elemental Mass Spec, Instrumentation, Mass Spectrometry
Application Code: Bioanalytical
Methodology Code: Mass Spectrometry
Thanks to the commercial introduction of columns packed with sub-2 µm particles and systems that can withstand pressure up to 1500 bar, highly efficient and/or ultra-fast separations can be relatively “easily” achieved today in reversed phase ultra-high performance liquid chromatography (RP-UHPLC). A new breakthrough came in 2007 with the commercial introduction of a new generation of columns packed with sub-3 µm superficially porous particles (SPP). Today, almost all providers of chromatographic consumables have their own range of columns packed with SPP. Today, this technology is widely used to decrease analysis times and/or improve resolving power, compared to columns packed with fully porous particles. Reversed phase liquid chromatography (RPLC) is the most widely used chromatographic mode, but hydrophilic interaction chromatography (HILIC) can be considered as an interesting alternative for the analysis of polar and/or ionizable compounds. Indeed, HILIC provides a very different elution order compared to RPLC and lower backpressure. Thus, higher flow rates with longer columns and smaller particle sizes can be used to achieve ultra-high performance in HILIC (UHP-HILIC). Another alternative to RPLC is supercritical fluid chromatography (SFC). In theory, SFC is a powerful strategy to perform green, fast and highly efficient separations. However, the sensitivity is often lower and the robustness of instrument quite limited. Recently, some providers have commercialized reliable SFC instruments as well as stationary phases packed with core-shell sub-3 µm particles or fully porous sub-2 µm particles, compatible with supercritical conditions. Then, there has been a regain of interest for this technology called ultra-high performance SFC (UHPSFC). In the present contribution, the possibilities offered by columns packed with small fully porous or SPP particles in RPLC, HILIC and SFC will be critically discussed, with a strong emphasis to pharmaceutical applications.
Ultra-High Performance Liquid Chromatography (UHPLC) is now a well established technique for analyzing pharmaceuticals, including small molecules and biotherapeutics. The key benefit of UHPLC over High Performance Liquid Chromatography (HPLC) is greater efficiency per unit time, enabling shorter analysis times without sacrificing resolution. This benefit arises from the use of highly efficient columns packed with < 3 micron particles. A wide range of UHPLC columns is now available, offering a variety of selectivities that may be screened to optimize separations. We will describe the most recently introduced UHPLC columns, including those packed with solid-core particles. These columns offer significant increases in efficiency per unit time compared to columns packed with fully porous particles. We will also describe recently introduced UHPLC columns designed for separations of biotherapeutics. Examples will be shown to demonstrate the use of these columns for separations of small and large molecule pharmaceuticals.

**Keywords:** Biopharmaceutical, HPLC, HPLC Columns, Pharmaceutical

**Application Code:** Pharmaceutical

**Methodology Code:** Liquid Chromatography
In the case of ion exchange, there are a number of factors that are of critical relevance to method development including proper choice of elution species and design of buffer systems. Often inappropriate choices in mobile phase will result in a large amount of pH hysteresis associated with the stationary phase such that the mobile phase pH is dramatically different than the stationary phase pH. Here we will review the best options for avoiding such issues and describe how to diagnose and correct stationary phase pH hysteresis issues.

In the case of mixed-mode stationary phases, optimization of the method is often complicated by the wide range of experimental parameters available to the analytical chemist. A general method development strategy will be presented, describing how to best approach method development when faced with a multitude of useful experimental operating parameters. Several example optimizations will be illustrated.

Optimization of size exclusion separations might seem to be unnecessary given the fact that size exclusion separations are defined by the stationary phase and independent of the mobile phase composition. But in fact, choosing the right pH can strongly influence both the separation and the stability of the stationary phase. Optimization of size exclusion separations will be illustrated with real world applications.

Keywords: HPLC, HPLC Columns, Method Development, Pharmaceutical
Application Code: Pharmaceutical
Methodology Code: Liquid Chromatography
Recombinant monoclonal antibodies are an important class of biological drugs and have been well characterized. In this presentation, I will share our recent results of using high resolution UHPLC methods to characterize antibodies variants (such as size, thiol and oxidation variants). These examples show UHPLC could reveal the antibody variants coeluting by HPLC methods. In conclusion, UHPLC is a powerful tool to characterize numerous heterogeneities of antibodies and serve as a useful quality control tool for antibodies drugs.
Development of new chemical entities (NCE) with high chemical and chiral purity is a regulatory expectation in new drug development. Today, more than half of the small molecule drug candidates are chiral compounds and it is not unusual to have complex molecules with 3 or 4 stereogenic centers which show high affinity for disease targets. For the process development of these “multi-chiral” molecules, numerous analytical methods capable of separating all stereoisomers (enantiomers and diastereomers) must be developed rapidly to assess and control the stereochemistry of raw materials, intermediates and the final active pharmaceutical ingredient (API). Achiral reversed-phase (RP) methods, used to assess the overall chemical purity assays, can typically be developed to monitor the diastereomeric content in a single run. In many cases, these achiral methods often become the primary quality control (QC) and stability-indicating purity assay methods. This paper describes the method development and QC strategies used for these complex multi-chiral drug molecules, which include:

• Adoption of a 3-pronged HPLC method development template approach
• Development of a single RP method using multi-segment gradients for determination of overall purity and diastereomeric content
• The use of UHPLC and software platform to expedite method development

These strategies will be described with actual examples used during clinical development of several new drug candidates.

Keywords: Liquid Chromatography, Liquid Chromatography/Mass Spectroscopy, Pharmaceutical, Quality Control
Application Code: Pharmaceutical
Methodology Code: Liquid Chromatography
Light Sources in Analytical Chemistry: Solid State Light Sources and Beyond

Structured Light from LEDs Enables Unique Spectrometer Design

Typical spectrometers are designed to maintain calibration independent of temperature (T), vibration, and age. Materials are chosen so that thermal expansion in mirrors, gratings, detectors, and support structure mutually compensate to maintain focus, dispersion, and wavelength calibration. Even so, T drift influences instrument precision not only through the effect on detector dark current but also due to changes in focus and wavelength alignment.

An alternative approach is to allow instrument dimensions to vary with T, but to calibrate alignment and dispersion at the moment of use. Doing so requires a reliable calibration source to be perpetually available. LEDs represent an example of such a portable reference. As characterized by Su et al.[1], the spectrum of phosphors in white LEDs shifts in predictable ways with T, and the emission wavelength of the GaN or InGaN blue pump LED emission also varies predictably with T, red-shifting by ~1 nm per 20K [2]. Thus, a white LED, while outputting light across the visible spectrum, can act as a wavelength standard if its T is known.

The presentation shows the design and characterization of a self-calibrating visible spectrometer. Complications due to wavelength-dependent detector response are illustrated. To broaden the range of problems addressable with LED-illuminated spectrometers, manufacturers are encouraged to develop multi-phosphor broad spectrum LEDs with UV pumps at ~ 350 nm and ~240 nm. Suggestions for broadly-applicable designs are presented, as are suggestions for why some obvious alternatives, such as stacks of narrowband LEDs, are less desirable.


Keywords: Instrumentation, Portable Instruments, Spectrometer, Spectrophotometry
Application Code: Agriculture
Methodology Code: UV/VIS
Abstract Text
This presentation will introduce the topic of the workshop "Light Sources in Analytical Chemistry: Solid State Light Sources and Beyond". Light sources in analytical chemistry are mainly associated with optical detection, encompassing many uses from sensors to absorbance and fluorescence detection in flow systems such as in flow injection analysis, liquid chromatography, capillary electrophoresis and other electromigration separation techniques. Some of the most exciting advances will be discussed including progress in deep-UV LEDs with commercially available UV LEDs making their way below the 250 nm range. Examples include photometric detection in capillary chromatography with a 235 nm UV-LED of analytes including iodide but also nitrate and nitrite. Further analytical uses other than optical detection will be examined in areas including microscopy, imaging, photolithography and photopolymerisation. New advances in the area of solid state light sources and specifically LEDs including some key non-analytical developments will be pointed out and the future of the light sources dubbed “light sources of the 21st century” will be discussed.

Keywords: Capillary Electrophoresis, Chromatography, Detector, Flow Injection Analysis
Application Code: General Interest
Methodology Code: New Method
Mid-infrared (MIR; 3-15 µm) sensor technology is increasingly adopted in environmental analysis, process monitoring, and biodiagnostics owing to the inherent molecular specificity. Thereby, discrimination of molecular constituents at trace concentration levels in condensed and vapor phase media is enabled. Recently emerging strategies taking advantage of innovative waveguide technologies combined with tunable quantum cascade and interband cascade lasers (QCLs, ICLs) facilitate compact yet robust MIR chem/bio sensors and diagnostics. Consequently, applications such as advanced breath analysis for disease detection or therapy progress monitoring, or on-site mycotoxin analysis in food and feed are readily enabled.

Keywords: Bioanalytical, Infrared and Raman, Laser, Sensors
Application Code: General Interest
Methodology Code: Vibrational Spectroscopy
Fiber optic devices are increasingly used in structural health monitoring, but also have many uses in chemical sensing. In the past decade we used fiber-optic devices for measurements of optical absorption, fluorescence refractive index, pressure, and strain. An optical cavity embedded into a telecom-grade optical fiber is very sensitive to strain and temperature. The quality of the transducer is demonstrated by using the system as a pickup for musical instruments, i.e. for recording sound distortion free and with a high frequency bandwidth. In a series of experiments we demonstrated that the “Photonic Guitar” pickup has a superior frequency response and far-superior signal-to-noise ratio over existing pick-up technology. Photoacoustic spectroscopy can be conducted using similar fiber cavities as strain sensing elements. These instruments are capable for detecting small changes in analyte concentration through the absorption of intensity-modulated laser light.

Keywords: Fiber Optics, Instrumentation, Monitoring, Photoacoustic
Application Code: Process Analytical Chemistry
Methodology Code: Physical Measurements
Reversed-phase HPLC is widely used for separation and analytical analysis of many complex mixtures. Unfortunately, there are mixtures that are not well separated by HPLC leading to incomplete analytical analysis. An alternative separation technique maybe required such as SFC (supercritical fluid chromatography) to effect a complete separation of many mixtures. In addition, SFC can be utilized as an orthogonal separation technique to HPLC for many separations. SFC provides many unique features to produce high speed “green” separations. These unique features suit SFC well to the separation of chemical mixtures containing of wide range of polarities. The chemical analysis of agricultural products, foods, beverages and nutritional supplements are a particularly difficult challenge. These materials display a wide range of chemical polarities, a high degree of chemically complexity and variety of functional chemical groups. However, with the proper stationary phase high performance SFC possess the capabilities to tackle the most difficult separations. The goal of this study is the present strategies for matching the best stationary phase for the optimal SFC separation agricultural products, foods, beverages and nutritional supplements. We will provide examples of agricultural products, foods, beverages and nutritional supplements separations that show the benefits of stationary phase matching. We will demonstrate how these various stationary phases can provide high resolution SFC separations over a wide variety of conditions.

Keywords: Agricultural, Food Science, Natural Products, Supercritical Fluid Chromatography
Application Code: Food Science
Methodology Code: Supercritical Fluid Chromatography
The US market for dietary supplements continues to grow with estimated sales of over $27 billion in 2015. At present, FDA rules for dietary supplements are limited to misbranding and safety but tighter regulation is expected to continue in the future and include areas such as manufacturing and efficacy. In order to assist manufacturers and testing labs with this increased complexity of tests we propose an efficient way to extract and analyze various dietary supplements in one complete SFE/SFC/LCMS system. We will demonstrate the efficiency and sensitivity of the SFE/SFC/LCMS system with analysis of omega3 fatty acids in fish oil capsules.

Abstract Text

Keywords: Automation, Food Science, Liquid Chromatography/Mass Spectroscopy, SFC
Application Code: Food Safety
Methodology Code: Supercritical Fluid Chromatography
Pesticide residue is a common concern for commercial spice manufacturers. Our objective is to develop a methodology by which we can detect pesticide from food products in a single instrument. Typically, analysis consists of sample grinding, some form of extraction technique followed by analytical analysis. This talk will discuss the means by which we utilize a single instrument to perform all these functions, except for the sample milling. We will utilize samples of commercially available samples of various pepper, including micro encapsulated formulations. For analysis we will demonstrate the efficiency of SFC with CO2 and modifiers for extraction, and SFC/MS for analysis.

Keywords: Chromatography, Food Contaminants, Pesticides, Supercritical Fluid Chromatography
Application Code: Food Contaminants
Methodology Code: Supercritical Fluid Chromatography
### Abstract Text

Traditional analysis of lipophilic vitamins and cholesterol in food requires various extraction and chromatographic methods which can be time-consuming and require the use of large amounts of solvents in some cases. For example, the current approach for multiple analytes may be illustrated by the use of numerous methods: cholesterol by GC-FID (AOAC 994.10); β-carotene by HPLC-FLD (AOAC 2005.07); retinol by HPLC-UV/VIS (AOAC 2001.13); vitamin E by GC-FID (AOAC 976.26); vitamin D by HPLC-UV/VIS (AOAC 981.17); and vitamin K by HPLC-FLD (AOAC 999.15). The aim of this study was to investigate the use of an integrated approach using SFE online with SFC-MS/MS. Validation results and application to various food matrices will be presented.

### Keywords
- Food Science
- SFC
- SFE
- Tandem Mass Spec

### Application Code
- Food Science

### Methodology Code
- Supercritical Fluid Chromatography
The analysis of numerous water-soluble micronutrients in food using a unified method can be very challenging due to the high hydrophilicity of the compounds and variability in polarity. Furthermore, the range in concentration can be wide from low trace levels to percentage levels, depending upon the food matrix. The challenge with many of the B-vitamins and Choline is that they naturally occur in conjugated forms, which require de-conjugation using acids and/or enzymes prior to extraction and analysis. Due to the challenges with the analysis of these compounds, microbiological methods are still used for analysis. Currently nutritional analysis of water soluble vitamins and choline is performed as follows: Vitamin C by HPLC-FLD (AOAC 984.26); Vitamin B1 (Thiamine) by HPLC-FLD (AOAC 957.17); Vitamin B2 (Riboflavin) by HPLC-FLD (AOAC 970.65); Vitamin B3 (Niacin) by HPLC-UV/VIS (AOAC 981.6); Vitamin B5 (Pantothenic Acid) by microbiological assay (AOAC 945.74); Vitamin B6 (Pyridoxine) by microbiological assay (AOAC 961.15); Vitamin B9 (Folate) by microbiological assay (AOAC 2004.05); and Choline by spectrophotometric assay (AOAC 999.14). These micronutrients may also be analyzed by LC-MS/MS, but this methodology often suffers from matrix ionization suppression effect from co-extractives in food. SFC-MS/MS has the potential for simultaneous analysis of water soluble micronutrients in a single method. In this study, 14 compounds were analyzed by two approaches in order to compare the suitability of either LC/MS/MS or SFC-MS/MS for an integrated methodology.
In the present work the carotenoids composition of a sample belongs to the Solanaceae family, specifically Capsicum was analyzed. Capsicum, originates from tropical and humid zones of Central and Southern America, is one of the oldest and most popular vegetable and spice in the world, and includes peppers. The research is focused on the development of an on-line method coupling supercritical fluid extraction (SFE) and supercritical fluid chromatography (SFC) for the detailed characterization of the carotenoid composition of a habanero red sample. The on-line nature of the system, compared to off-line approaches, improves run-to-run precision, enables the setting of batch-type applications, and reduces the risks of sample contamination. Supercritical carbon dioxide (CO2) presents unique characteristics, which make it an excellent solvent. CO2 shows a relatively high density and consequently, a high solvation power. It presents low viscosity and high diffusion coefficient, which allow fast extraction. From the environmental point of view, the use of supercritical CO2 for both purposes, SFE and SFC, greatly reduces the use of organic solvents. The density of the supercritical fluid can be easily handled by changing its pressure and/or temperature, changing the solvent strength. The same considerations can be made regarding the SFC, thus obtaining rapid analysis characterized by high resolution. Finally, the MS detector due to its high sensitivity and selectivity allows the identification and structural analysis of targeted and untargeted compounds.

Keywords: Chromatography, Mass Spectrometry, SFC
Application Code: Food Identification
Methodology Code: Supercritical Fluid Chromatography
Bioanalytical - Fluorescence/Luminescence Techniques

Development of High-Throughput Instrumentation for Single-Cell Viscometric Analysis Via Fluorescence Anisotropy

Cellular viscosity is of key interest when studying cell morphology, cell motility, and intracellular protein-protein interactions. Traditionally, fluorescence spectroscopy has been used to study microenvironment viscosities within the cell via fluorescence correlation spectroscopy and molecular rotors; however, these techniques are limited by long data acquisition times, are spatially restricted, and have low cell throughput. Presented in this work is the development of a high-throughput, robust instrumentation that is used to study intracellular viscosity via fluorescence anisotropy. Fluorescence anisotropy is not limited by fluorescent probe concentration and can be used to empirically determine the viscosity of a cell sample. A microfluidic device hydrodynamically focuses cells over a stationary excitation source and the polarized emission is detected in two planes of polarization, parallel and perpendicular to the excitation source. This proposed instrumentation will be used to ascertain baseline distributions cell viscosities and their variation in response to two major biological events: cell differentiation and cellular response to apoptosis under normoxia and hypoxia.

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Abstract Text

Abstract Text

Date: Thursday, March 09, 2017 - Morning
Time: 08:30 AM
Room: W175a
Abstract Text
Bioanalytical (BL)-based assays are widely used as [i]in vitro[/i] and [i]in vivo[/i] reporters of biologically relevant substances, gene expression, and tumor progression, among others. Among the available systems, firefly bioluminescence shows the highest emission efficiency ([i][\text{BL}][/i] = 0.41). Although bioluminescence-based assays show much lower background signals than fluorescence-based assays and are applicable for more sensitive [i]in vivo[/i] imaging, their application is limited due to a lack of luciferins emitting in the near-infrared (NIR) wavelength (650~900 nm), where tissue is more light transparent.

In this work, novel firefly luciferin analogues modified with allyl groups at the carbon C-7 position of the benzothiazole core were developed. The terminal olefin of allyl groups is for example available for further modification as a linker, or the allyl groups can also be converted to aldehyde groups, which can then react with various functional groups. Therefore, it is considered that allyl modification of firefly luciferin leads to useful possibilities, such as the development of various analogues and labeling applications by using allyl luciferin as a platform. Additionally, we hypothesized that this steric modification would result in red-shifted bioluminescence emission due to the conformational change that creates an extremely hydrophobic microenvironment in the luciferase active site.[i][1] As expected, the developed luciferin showed 45 nm red-shifted emission, presumably due to the change in the interaction between the luciferin and amino acid residues at the active site of the luciferase enzyme. Additionally, live cell assays of the allyl-luciferin with some beetle luciferases were performed. Surprisingly, the allyl-luciferin showed luciferase-selective response. This result suggests that this synthetic luciferin can be applied to luciferin-luciferase based sensing for [i]in vivo[/i] imaging, such as for multiple BL assays.

Based on these findings, five types of allyl modified luciferin analogues were newly developed. These analogues exhibited longer emission wavelength and extended the bioluminescence emission into the NIR wavelength with wild type luciferase. We believe that these new synthetic luciferins contribute to the expansion of bioluminescence imaging applications both [i]in vitro[/i] and [i]in vivo[/i].


Keywords:  Bioanalytical, Chemiluminescence, Imaging, Near Infrared
Application Code:  Bioanalytical
Methodology Code:  Fluorescence/Luminescence
Chiral interfaces are ubiquitous in biological systems. Despite this, there are relatively few methods for chiral interface-specific characterization. Fluorescence optical rotary dispersion (FORD) is a newly proposed chiral-specific spectroscopic method for probing structure at biological interfaces. FORD may be conducted using common equipment from a fluorescence microscope. The chiral specificity of the FORD measurement is completely electric dipole allowed suggesting higher sensitivity than traditional absorbance circular dichroism methods. The proposed method provides an explanation for high linear dichroisms observed in previous single-molecule chiral-specific studies. FORD may provide methods which suppress background fluorescence in studies of biological interfaces, as the detected signal requires both polar local order and interfacial chirality. Furthermore, extension of the framework to surface enhanced Raman optical activity is ongoing, and provides a novel mechanism for chiral specific measurements.

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Graphene materials, the famous two-dimensional nanomaterials, recently have attracted enormous interests in nanoptics and material research. The combination between graphene materials and plasmonic structures has shown novel optical properties. In addition, the unique ability of graphene materials to selectively adsorb molecules through pi stacking interaction makes it an attractive candidate for biosensing research. Considering that surface plasmon coupled emission (SPCE) holds great promise to improve the fluorescence detection ability and graphene material has shown good compatibility to SPCE system, herein we demonstrate a signal enhancement of graphene oxide (GO) assisted SPCE on gold nanofilm for sensitive immunoassay. A sandwich structure with GO introduced between the dye layer and the gold nano-film for SPCE measurement was established. Compared with free space emission without GO, the enhanced factors was 25 for GO-assisted SPCE. Taking the advantages of this enhanced GO-assisted SPCE system, an immunoassay for sensing human IgG was established with a limit of detection as low as 0.006 ng/ml. We also developed a novel aptasensor utilizing a sandwich substrate successively deposited by gold nano-film, silica isolation layer and GO to connect a fluorophore labeled DNA aptamer. In this design, aptamer and substrate together constituted interfacial molecular beacon, without the need to involve complicated configuration such as hairpin structure in molecular design; a tiny configuration change caused by biorecognition to distance fluorophores from GO surface sensitively induced signal response. The introduction of graphene materials in SPCE offers great promise in biosensing detection.

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Keywords: Biosensors, Fluorescence, Molecular Spectroscopy, Nanotechnology
Application Code: Bioanalytical
Methodology Code: Fluorescence/Luminescence
A Label-Free Aptamer-Fluorophore Assembly for Highly Sensitive and Specific Detection of Cocaine

We have developed a rapid and specific aptamer-based method for one-step cocaine detection utilizing the cocaine-mediated displacement of the fluorophore ATMND from the anti-cocaine aptamer 38-GC. We obtained a linear range of 0–8 uM and a limit of detection (LOD) of 200 nM within 20 seconds. The LOD was 50-fold better than most existing aptamer-based systems and comparable to sensitive assays that require enzymatic amplification.

Upon the successful demonstration of the sensor platform, we validated the assay using different sample matrices including different beverages (tea, soda, Gatorade, and various alcoholic drinks) and biofluids (urine, serum and saliva). The results showed that the assay obtained LODs of 460, 900 and 520 nM in 2.5% urine, 2.5% serum and 5% saliva, respectively. The LODs are equivalent to 18.4, 36 and 10.4 uM in undiluted urine, serum and saliva, respectively. The assay also demonstrated high target specificity against the major cocaine metabolite, benzoylecgonine.

Keywords: Bioanalytical, Biological Samples, Biosensors, Fluorescence
Application Code: Bioanalytical
Methodology Code: Fluorescence/Luminescence
“Steric trapping” is a method that links binding of monovalent streptavidin (mSA) to unfolding of a biotinylated membrane protein (MP). It allowed the measurements of high affinity protein–protein interactions and thermodynamic stability of polytopic helical MPs in a native environment, which had been difficult to achieve using more conventional methods. In this study, we further advance the steric trapping strategy for more general application by developing versatile biotin probes possessing spectroscopic reporters that are sensitized by mSA binding or protein unfolding. By applying these methods to the Escherichia coli intramembrane protease GlpG, we elucidated a widely unraveled unfolded state, subglobal unfolding of the region encompassing the active site, and a network of cooperative and localized interactions to maintain stability. Because of the accurate determination of thermodynamic stability, this method can provide a significant tool for the study of MPs.
Enzyme-linked immunosorbent assay (ELISA) has become one of the most popular immunoassays for detecting and quantifying the concentrations of biomarkers and pathogens in the past decades. Due to its high specificity and reproducibility, this technique is widely used in medical diagnosis and basic biological research. However, the traditional 96-well-plate ELISA still suffers from several notable drawbacks such as long assay time (~6 hours), burdensome procedure and large sample/reagent volume (~100 µL). These disadvantages limit traditional ELISA’s application in rapid clinical diagnosis and semi-real-time prognosis of some fast-developing immunological diseases such as acute organ rejection. In order to solve these problems, we developed a glass-capillary based microfluidic ELISA system, which performed a sandwich ELISA reaction within a 1mm² cross-section area silica capillary. Benefiting from the high surface-to-volume ratio of the capillary, this technique significantly reduced sample volume to around 20 µL and also reduced the total assay time to less than 30 minutes. The readings of antigen concentrations were taken by photo-imaging method with chemiluminescent substrate, thus reduced the total measurement time from 15-30 minutes to less than 1 minute (after adding substrate). In comparison to conventional ELISA, the microfluidic ELISA can considerably shorten the total assay time (~12-14 times faster), lower the sample volume (5 times smaller) and enhance the sensitivity (from around 10pg/ml to around 1pg/ml), while maintaining good dynamic range (~3 orders of magnitude) and good stability. The properties of microfluidic ELISA and its future application in clinical practice will also be discussed.

Keywords: Chemiluminescence, Imaging, Immunoassay, Quantitative
Application Code: Bioanalytical
Methodology Code: Fluorescence/Luminescence
Electrophysiological recording is a powerful and informative tool for studying ion channel activities. Electrophysiology can be used for a wide range of studies such as understanding ligand binding kinetics, selective transport of ions and the role of ion channels in their native environment. However, obtaining electrophysiological recording is labor intensive, resulting in low throughput, thus limiting its applications. To overcome this problem, microfluidic array devices can be used for electrophysiological measurements to allow parallel testing, improving the throughput. In this study, a surface-modified glass/PDMS pneumatic valve was evaluated as a main component for a microfluidic array device tailored for electrophysiological measurements. Current existing array devices utilize one electrode pair to measure signals coming from each cell and thus require the use of multiple patch clamp amplifiers. The incorporation of high seal resistance valves into the array device allows only the desired electrophysiological signal to be detected by the patch clamp amplifier, enabling parallel experiments with one patch clamp amplifier and greatly improving the efficiency. To achieve the desired high seal resistance of the valve, surface modification with perfluorodimethylchlorosilane was performed to the glass components to increase the interaction between glass and PDMS. The modified valves exhibited hundred-fold increase in seal resistance, along with millisecond opening time and one-second closing time. The steady-state noise level was in pico-amp regime. Furthermore, the modified valve was used to successfully raster on and off states to acquire hemolysin signal during electrophysiological measurements, which demonstrated the utility to be incorporated into electrophysiological microfluidic array devices.
Kinetic analysis of enzymes has typically been limited to bulk measurements, which hinders our perspective of how enzymes work at cellular length scales where they reside in confined and crowded environments. Micro-total analysis systems (μTAS), specifically lab on a chip (LOC) devices provide a means to study enzyme kinetics at the micro and nano scale, giving more realistic kinetic data. Here, kinetic analysis experiments were carried out using an array of nanofluidic channels in a borosilicate glass LOC device to simulate confinement and crowding at cellular length scales. Channels measuring 100 nm x 40 μm were wet etched directly into the glass and annealed at the transition temperature of borosilicate. Here, we propose a method to detect glucose via fluorescence using a two-enzyme system of glucose oxidase (GOx) and horseradish peroxidase (HRP). Glucose solutions are introduced electrokinetically into the LOC device along with the GOx yielding hydrogen peroxide (H₂O₂) and gluconic acid. From here, the products are moved downstream where the H₂O₂ can react with non-fluorescent Amplex Red (AR) in the presence of HRP and yield water and fluorescent resorufin. Fluorescence can be correlated to the amount of H₂O₂ produced, and thus to the amount of glucose initially present.
The free, or non-bound, fraction of a drug in blood is often the biologically active form. Ultrafast affinity extraction is one approach that has been used to measure this form. Alpha1-acid glycoprotein (AGP) is one of the most important acute phase proteins and is the principal binding protein for basic and neutral drugs in serum. AGP was used in this study as a ligand to prepare affinity microcolumns for use in ultrafast affinity extraction. These columns were then used for quantitatively extracting analytes/drugs in sub-second time domain and for examining the binding and dissociation kinetics of various drugs with soluble AGP. Chromatographic parameters such as column size and flow rate were optimized during the measurement of free drug fractions to avoid interference from dissociation of the bound form in a sample. Various drug targets were studied, including propranolol, imipramine, lidocaine, verapamil, chlorpromazine and disopyramide. The free drug fractions and equilibrium constants that were determined by this approach were comparable to the results of reference methods. The study is now being done with the clinical samples to better understand how the clinical changes in serum proteins affects the drug protein binding interactions. This approach can be extended to the screening and rapid analysis of other solute-protein interactions of biomedical interest and could lead to the creation of improved analytical methods for such studies.

Keywords:  
Bioanalytical, Chromatography, HPLC, Separation Sciences

Application Code:  
Bioanalytical

Methodology Code:  
Separation Sciences
Noninvasive blood glucose measurements have been demonstrated in an animal model where near infrared spectra were collected from skin during glucose transients.\cite{1-5} In these experiments, calibration models derived from the net analyte signal (NAS) were used to predict the concentration of glucose within the skin matrix. The NAS corresponds to the component of the analyte spectrum that is orthogonal to background (non-analyte) sources of spectral variance derived from the sample. Unlike other multivariate calibration methods such as partial least-squares (PLS), the NAS method is not subject to spurious correlations because the fundamental calibration function is derived directly from the glucose spectrum.

Our research goal is to expand NAS-based noninvasive glucose measurements to human subjects. The effectiveness of any NAS prediction depends on the existence of background spectra that describe the non-analyte dependent spectral variance of the sample matrix. In past animal model studies, such background spectral variance was determined from a principal component analysis (PCA) of skin spectra collected while the concentration of glucose was held constant over a period of hours with an anesthetized animal. Our challenge is to generate a relevant set of background spectra for non-anesthetized human subjects.

This presentation will focus on noninvasive near infrared spectra collected from human subjects during periods of constant and changing blood glucose concentrations as a means to characterize non-glucose dependent spectral variance. Variations in human skin background spectra will be presented and the impact of these variations on the reliability of NAS calibration models for glucose will be discussed.


Keywords: Bioanalytical, Chemometrics, FTIR, Near Infrared
Application Code: Bioanalytical
Methodology Code: Near Infrared
SFC has been applied for separation and purification of small molecules for decades. The low viscosity and high diffusivity of supercritical CO2 provide higher separation speed and lower back pressure than HPLC. Can SFC-MS/MS be used for higher throughput small molecule routine bioanalysis in drug discovery? How does it compare to LC-MS/MS? More than 30 small molecule compounds, commercially available or Genentech synthesized, were evaluated using 6 achiral columns on a Shimadzu SFC system. The supercritical condition for CO2 was set at 100 bar and 40°C. Four mobile phase modifiers, from acidic to basic, were evaluated. A gradient of 10% to 40% of mobile phase modifier with 3ml/min flow rate was used and the total run time was 1 min. Compounds were detected using a Sciex Qtrap6500 triple-quadrupole mass spectrometer in ESI positive mode. Biological samples were precipitated with acetonitrile. Supernatant was injected to a SFC-MS/MS system and then reinjected to a LC-MS/MS system for comparison purposes. With combination of 6 columns and 4 mobile phase modifiers on >30 compounds, 75% of more than 700 chromatograms showed reliable and consistent peak shape. However, by using certain columns and mobile phase modifiers, the success rate can be increased to 94%. Matrix effect and carryover on SFC were comparable to those on HPLC. Sensitivity and standard curve linearity were compared between SFC and HPLC. Detailed correlation between two sets of concentration data produced by SFC-MS/MS and LC-MS/MS will be presented.

Keywords: Bioanalytical, Drug Discovery, Mass Spectrometry, Supercritical Fluid Chromatography
Application Code: Bioanalytical
Methodology Code: Supercritical Fluid Chromatography
MicroRNAs (miRNA) are non-coding RNAs, ~22nt in length, that bind to target mRNAs to inhibit translation or induce degradation of target transcripts. They are potential markers for diagnosis of diverse diseases including cancers, coronary disease, and Alzheimer’s disease. Compared to the widely used nucleic acid-based disease markers, mRNAs, miRNAs have been found to be more closely related to disease stages and are tissue specific. Moreover, miRNAs can be released into the circulation system and stably present at levels detectible by sensitive techniques like RT-PCR.

MiRNAs found in the extracellular environment of circulatory fluids are protected from the abundant ribonucleases in that environment by various types of carriers. These carriers can be the vehicle, by which the miRNA is secreted from cells which can indicate their origin, how they are secreted and transported to other cells for cell-cell communication. While miRNA secretion by malignant cells could be the consequence of dysregulation of cellular pathways, exportation and uptake could be related to tumor progression and metastasis. Therefore, it is more informative to present the expression levels of miRNAs with association to their carriers, compared to simply quantifying their overall quantities.

Microfluidic technology is a currently investigated field for improving sample work-flow, increasing the rate of sample processing and analysis, and reducing sample consumption. Microfluidics allows for greater sample manipulation without possible contamination or sample loss associated with bench top techniques. Microfluidic technology towards distribution profiling of serum miRNA carriers also provides the foundation of point-of-care devices with integrated detection. The currently developed technique allows for the isolation of 3 discrete sub-fractions of miRNA carriers with minimal cross-contamination between carriers; these carrier fractions are proteins, lipoprotein complexes (HDL/LDL), and exosomes.

**Keywords:** Bioanalytical, Biological Samples, Nucleic Acids, Separation Sciences

**Application Code:** Bioanalytical

**Methodology Code:** Separation Sciences
Surface-enhanced Raman Scattering (SERS) has emerged as a powerful characterization tool in a large number of applications, including pathogen detection, materials analysis, and food safety. Nonetheless, concerns about the quantitative reliability of the measurements and sample throughput remain barriers to its widespread adoption. This presentation details the results of work focused on delineating the underpinnings of the origins of measurement reproducibility and on efforts to increase sample throughput for heterogeneous immunoassays utilizing SERS as a readout mechanism. With respect to measurement reproducibility, the results from both a theoretical model and experimental studies on the impact of sampling error on accuracy and precision show that the size of the focused laser spot has an important but little recognized role on the reliability of measuring analytes bound to a surface. This sampling error is manifested when a measurement of the surface concentration in an area over or underestimates the composition of the larger, more representative sample. Drawing on the results of these studies, a 96-well microplate reader was designed that uses six channels to read out a microplate in 60 s. By using a simple spectral internal standard, this system can achieve a measurement repeatability error of ~1%. Examples that demonstrate the high throughput capability of the system will also be given.
Many health-related conditions are difficult to diagnose in the early stages of disease. New sensing technologies that are sensitive, selective, rapid, label-free, have multiplexing capabilities, and require little to no sample processing is an area of need. Our group is developing surface-enhanced Raman (SERS)-based sensors for the detection of biomarkers of disease in non-invasively collected biofluids. SERS is a highly sensitive, vibrational spectroscopy that can identify small molecules at low concentrations and provides fingerprint-like spectra. SERS-based sensors fulfill the requirements of: little to no sample processing; being rapid and label-free; and designed to include multiplexing capabilities. Additionally, with the development of portable Raman spectrometers, SERS measurements can be performed in the field in real-time. We present progress on the detection of biomarkers of neurological activity in non-invasively collected biofluids with SERS-based sensors.

Keywords: Biosensors, Raman Spectroscopy, Surface Enhanced Raman Spectroscopy
Application Code: Bioanalytical
Methodology Code: Vibrational Spectroscopy
A variety of recent, high performance assay formats exploit quantitative electrochemical detection of DNA hybridization or aptamer-target binding using square wave voltammetry (SWV). The voltammetric data consists of both Faradaic (signal) and non-Faradaic (baseline) components; subtracting the former from the latter produces a corrected, concentration-dependent signal. Using standard potentiostats, this baseline correction must be performed post-experiment rather than during the experiment, imposing upper limits on sensitivity settings when non-Faradaic (capacitive) currents are high. Here, we show that a differential potentiostat (DP) circuit allows on-board analog subtraction, minimizing amplification of non-Faradaic current as well as decreasing white noise. By collecting both signal and baseline electrode measurements simultaneously with a pair of working electrodes, the analog signal is differentiated by an on-board instrument amplifier and quantified. Circuit simulations were performed to optimize the design, a daughterboard PCB was then fabricated and populated with components, and these circuits were coupled to an open source, low-cost, USB powered potentiostat (DStat). After initial testing, the DP was evaluated by a hybridization assay of methylene blue tagged DNA (MB-DNA). SWV analysis was performed on baseline electrodes (no MB-DNA) and signal electrodes (hybridized MB-DNA) by both a standard potentiostat (Gamry Reference 600) and the DP circuitry. As represented in Figure 1, studies with 5 electrode pairs showed a white noise reduction of 4.6 ± 1.5 fold and a near complete removal of non-Faradaic current. This DP circuitry should set the stage for further increases in sensitivities in surface-confined, SWV-based assays, particularly those involving DNA hybridization or aptamer-target binding.
Electrochemical measurements are difficult to make in complex biologic solutions because as soon as the electrode is placed in solution, irreversible biofouling takes place. Recently we developed a means to make electrochemical measurements in such solutions by using nanoporous gold electrodes. These electrodes are composed of nanometer sized pores that serve as a sieve, screening out large biomolecules but allowing small redox molecules to interact with the pristine surface (Collinson et al Analytical Chemistry 2013). Such electrodes are ideal tools to measure the open circuit potential (OCP) of complex solutions including blood and plasma. The focus of this talk will be on the fabrication and characterization of gold and platinum nanoporous electrodes and their ability to successfully measure the redox potential of blood and other complex solutions. Particular attention will be given to the determination of the sensor effective redox couples that influence the value of OCP at these electrodes as well as the measurement of the OCP of stored blood products.
Fast-scan cyclic voltammetry (FSCV) and amperometry, coupled to carbon-fiber microelectrodes, are powerful tools for measuring real-time chemical dynamics in live tissue and at single cells. These approaches offer precise temporal and spatial resolution with electrodes typically fabricated in a cylindrical or disk geometry. FSCV typically employs cylindrical electrodes in live-tissue preparations, while amperometry is performed with disc electrodes at single cells. The cylinder geometry exhibits greater sensitivity due to a larger electroactive surface area, whereas the disk geometry offers greater spatial resolution with decreased surface area. However, several applications that sample from a small area would benefit from enhanced sensitivity, including research on exocytosis at single cells. Herein, a plasma-etching procedure is described for creating carbon-fiber electrodes in a cavity geometry with precise control of cavity depth. Fabrication of these electrodes is simple and inexpensive, and a significant step forward for electrochemical experiments at preparations including, but not limited to, single cells. These cavity electrodes exhibit enhanced sensitivity compared to disk electrodes, despite similar overall dimensions. This arises from the heightened surface roughness of the etched carbon-fiber, as studied via SEM, as well as restricted diffusion of analyte away from the electrode surface. These electrodes are compared to disk microelectrodes at single cells, detecting more molecules per release event. This suggests that efforts to determine the dynamics of vesicular fusion may currently rely on incomplete information. Cavity electrodes will provide an invaluable tool to more fully detect exocytotic events, offering more reliable data and potentially improving models of quantal release.

Keywords: Bioanalytical, Electrochemistry, Electrodes, Neurochemistry
Application Code: Neurochemistry
Methodology Code: Electrochemistry
Chloride, at an extracellular concentration of 105 +/- 5 mM, is the most prevalent anion in human blood. Classical potentiometry with ion-selective electrodes is very convenient for the measurement of simple ions such as chloride. However, several ions found in blood interfere with chloride measurements due to their higher lipophilic nature. For example, salicylate, resulting from the metabolism of aspirin, can reach up to 2 mM, and is approximately 1,000 times preferred over chloride using ion-selective electrodes. The objective has been to develop a method for measuring a smaller hydrophilic ion in the presence of a larger lipophilic ion, using chloride and salicylate as examples of each. Our asymmetric cellulose triacetate (CTA) membranes are made of a pure CTA barrier layer and an electrically active component containing sensing layer, further modified by hydrolyzing one side of the membrane with sodium hydroxide, creating physical pores and a more hydrophilic membrane surface. This in turn allows for faster passage of small ions like chloride into the membrane, while retarding the movement of larger ions like salicylate, in a process known as kinetic discrimination. Use of pulsed chronopotentiometry, in which current is applied, is required so that the small concentration of the lipophilic anions that reach the sensing surface is depleted via extraction. This alleviates the interference of lipophilic anions on the measurement of smaller hydrophilic anions. If successful, we plan to apply this methodology towards the measurement of other small hydrophilic ions of biological and environmental importance in the presence of various relevant lipophilic anions.
Protein nanopore-based stochastic sensing represents a powerful technique in sensitive single molecule analysis impacting fields ranging from single molecule bioanalytical studies to next-generation DNA sequencing. The detection method is, at its basis, a resistive-pulse technique which relies on changes in the conductance of, or current through, a protein nanopore when a molecule of interest translocates through the pore. The majority of studies to date rely on the isolation of a single protein channel embedded in a highly-resistive lipid bilayer membrane for ease of data collection and analysis. The addition of multiple channels has the ability to increase sensor throughput and sensitivity but at the cost of signal complexity. Here, we present a relatively straight-forward method to quantitatively analyze single molecule binding data with multiple embedded channels. To demonstrate the ability to quantitate target concentration, we employed a model system comprising the protein channel [alpha]-hemolysin ([alpha]HL) and the target analyte heptakis(6-O-sulfo)-[beta]-cyclodextrin (s7[beta]CD). We find that the binding rate for each channel follows a Poisson process. When multiple channels are present we find that the binding rate is additive following the superpositioning property of Poisson processes, assuming each channel is independent. Knowing the number of channels present allows for quantitative determination of bulk s7[beta]CD concentration with higher sensitivity than using single channels. The ability to readily quantitate analyte concentration using the signal from multiple channels facilitates the use of techniques where using one channel becomes impractical or limiting such as scanning ion conductance microscopy using protein channels.
Osteoarthritis is a debilitating condition of synovial joints, leading to pain and severe limitations in mobility. The condition proceeds via initial mechanical stress combined with low grade inflammatory processes, and over time cartilage is lost. Unfortunately, natural cartilage has limited regenerative capabilities, in part due to avascularity. For this reason, a proposed treatment to promote cartilage regeneration is delivery particles able to locally release specific cargo.

Nano-sized particles are highly suited as delivery vehicles due to their many advantages including tunability, adaptable surface chemistry providing sensing ability, high cargo loading and prolonged circulation time (1). They can be tailored to release cargo under selected environmental stimuli including response to chemicals mimicking intracellular environments (2), temperature, and the presence of biological compounds (3). Within the Stevens group we have developed nanoparticles able to release cargo in response to the presence of selected enzymes.

In general, release is monitored via fluorescently labelled cargo (2). This can, however, prove difficult to measure quantitatively, is often only taken at time-points and may not be suitable for all platforms. Being able to monitor the release in real-time, continuously and with high sensitivity would be beneficial (4). In this work an electrochemical protocol for real-time monitoring of cargo release from nanoparticles will be presented and preliminary findings shown. This study can be translated to compare cargo release from different particles and under different environmental conditions.

References:
Bioanalytical Electrochemistry

Carbon-Pyrenyl Nanostructures for Biosensing and Enzyme Electrocatalysis

The conductive large surface area with ease of functionalization of carbon nanotubes allowed the development of highly sensitive electrochemical immunosensors and electrodes featuring chemical or enzymatic catalysts yielding high electrocatalytic currents. In particular, conventional chemical treatment based and recently growing noncovalent functionalization strategies of carbon nanotubes have shown remarkable applications in the areas of electrocatalysis, biosensors, materials science, and renewable energy devices. New functionalization strategies to obtain fundamental understanding of carbon nanostructures are still evolving. We present here our findings on the influences of covalent versus noncovalent functionalization of carbon nanotubes in affecting enzyme electrocatalytic currents and sensitivity of serum insulin immunosensors. We additionally present carbon nanotubes-pyrene functionalized surfaces for ultrasensitive electrochemical detection of small molecule urine markers and large serum biomarkers.

Keywords: Bioanalytical, Biosensors, Electrochemistry, Electrode Surfaces

Application Code: Biomedical

Methodology Code: Electrochemistry
A potentiometric detection of the DNA hybridization with the use of polyaniline (PANI) composite materials has been studied. The PANI composite materials were prepared chemically with template technique using Nylon. The resulting PANI-Nylon films were used as prepared. The non-covalent immobilization of single stranded oligonucleotides (probe) was carried out.

The decrease of the indicator electrode potential was observed during the immobilization due to electrostatic interactions between positively charged PANI surface and negatively charged phosphate groups of oligonucleotide strands. The addition of the complementary stranded oligonucleotide (target) to the solution containing indicator electrode with immobilized probe leads to the increase of the indicator electrode due to the hybridization event occurring on the surface of PANI and leave the hybrid from the surface of the indicator electrode. The formation of the double helix and its release from PANI surface was proved with fluorescent spectroscopy.

The covalent immobilization of the probe causes the similar drop of the potential of the indicator electrode. Addition of the target gives the similar positive response however is not accompanied with the release of the duplex from the electrode surface. The response amplitude of the biosensor depends on the amount of bases in the immobilized target and does not depend on the amount of guanine bases in the sequence.

The experiments with synthetic polyanions have shown that the addition of the negatively charged polymer to the solution containing PANI as an indicator electrode causes minor response compared to that observed on probe immobilization. This fact indicates that interactions of single stranded oligonucleotides with polyaniline have rather specific nature than electrostatic.

Acknowledgement
Zhanna Boeva is expressing her gratitude to Jane and Aatos Erkko Foundation for the financial support.

Keywords: Biosensors, Nucleic Acids, Potentiometry
Application Code: Bioanalytical
Methodology Code: Electrochemistry
Quality control analysis of biochemically relevant pharmaceuticals is an important process in drug industry. Various chromatographic, spectroscopic, titrimetric, and electrochemical methods have been used in pharmaceutical industry for the estimation of active chemicals in drugs. In this study, we report a convenient, rapid, and cost-effective electroanalysis of oxidative pharmaceuticals as single 50 [micro]L drops on screen printed electrodes (SPEs) modified with carboxylated multiwalled carbon nanotubes (MWCNT-COOH). Acetaminophen (a pain-killer), Ascorbic acid and Nicotinamide adenine dinucleotide reduced form (NADH) [dietary supplements], and Nicotine (the active agent of anti-smoking pharmaceuticals) were analyzed in this study. Single drop electro-oxidation of the above compounds have demonstrated excellent analytical parameters, especially low limits of detection with high sensitivities in nA current per [micro]M analyte. We determined that chemical structure, molecular size, and polar/nonpolar properties contribute significantly to the interaction of a single drop analyte with the electrode surface to facilitate interfacial charge transport, and thus the oxidation current signals. Our approach was then tested with commercially available above pharmaceuticals and percentage recovery in the range of 92-99% was obtained. These successful findings suggest the applicability of single drop electroanalysis as a cost-effective and instant analytical tool for the quality assurance (QA) process in pharmaceutical industry, especially to determine the purity of an active chemical form of a drug.

Keywords: Biomedical, Biosensors, Electrode Surfaces, Pharmaceutical
Application Code: Pharmaceutical
Methodology Code: Electrochemistry
Here, we report for the first time the design and fabrication of a “green” electrochemical sensor cable of quantifying APAP in serum. The sensing strategy relies on the previously unexplored strong interaction between the APAP and designed molecule. The function groups of APAP were structurally associated to relative Arg via four hydrogen bondings. Taking advantage of this selectivity and high tenability of hydrogen bonding, we utilized functional layer, which immobilized on the graphene surface via electrostatic interaction as a driving force, to selectively capture APAP molecule and examined by multiple electrochemistry method. Here we utilized L-ascorbic acid as the reductant and Arg as the stabilizer to produce ‘green’ graphene from graphite oxide. The sensor showed a similar performance in serum as in buffer and obtained good recoveries as well. This proof of concept might provide a robust and highly selective approach for small molecule detection, promising the new separation-free sensor technique.
Biotechnology has seen a steady 5% annual growth from 2011-2016. This represents the ever growing need for the detection of newly developed drugs such as therapeutic antibodies. Our lab has pioneered a powerful approach using a short synthetic peptide (M.W. less than 2.2 kDa) as recognition element in lieu of antibodies and/or scFvs to develop an inexpensive, rapid, sensitive, specific and reusable label free immunosensor to detect a therapeutic antibody biomarker in human serum. The term “mimotope” has been coined for its description of small peptides which mimic the epitope of protein interfaces. Peptide mimotopes despite their short sequence can be very versatile in their applications including drug development, peptide libraries, and biosensors. Mimotopes offer significant advantages over ligands and antibody for biosensor development as they can exclusively mimic an epitope and retain a small size. This allows mimotopes to have a specificity comparative to antibodies, but the flexibility of ligands at a significant reduced cost since only a peptide needs to be synthesized rather than a protein. In this presentation, we will show examples for the design and selection of mimotopes to form self-assembled monolayer (SAM) and illustrate the peptide mimotope SAMs for their uses as molecular recognition elements for label free immunosensor development. We will further show the buffer and packing conditions on peptide SAMs (self-assembled monolayers), as well as incorporating the peptide SAM with an electrochemical impedance readout for a novel point of care diagnostic method.

Keywords: Bioanalytical, Chemically Modified Electrodes, Electrochemistry, Electrode Surfaces
Application Code: Bioanalytical
Methodology Code: Electrochemistry
### Abstract Text

Drugs of abuse such as methamphetamine elevate extracellular catecholamine (e.g. dopamine and norepinephrine) concentrations in the brain. Methamphetamine is a widely abused psychostimulant drug that effects catecholamine transmission in the brain mainly through promoting efflux of catecholamines by inhibiting and reversing their clearance mechanisms (e.g. transporter proteins). However, the precise mechanisms by which methamphetamine effect norepinephrine in comparison to dopamine release are still not well understood due to the limitations of conventional neurochemical techniques for measuring catecholamine concentrations which lack the spatial and temporal resolution necessary to monitor rapid fluctuations in catecholamine concentrations. Herein we used in vivo fast-scan cyclic voltammetry to study the effects of increasing doses of methamphetamine on dopamine and norepinephrine release in the brains of behaving rats and compared their differences. These results highlight in real-time how methamphetamine effects extracellular catecholamine release in a dose-dependent manner and further highlights the role of norepinephrine in methamphetamine abuse.

### Keywords
- Drugs
- Electrochemistry
- Neurochemistry
- Voltammetry

### Application Code
- Neurochemistry

### Methodology Code
- Electrochemistry
Abstract Text
Psychological disorders, including attention-deficient/hyperactivity disorder (ADHD), are on the rise while effective treatments remain elusive. Current treatments for ADHD include amphetamines, which are believed to influence serotonin signaling in the brain. However, the influence of pharmaceutical drugs on the serotonin system’s in vivo neurochemistry is not well understood, due to a lack of analytical techniques capable of monitoring low concentrations of neurotransmitters at the fast time-scales relevant to in vivo neurotransmission. In this work, we employ fast-scan cyclic voltammetry to monitor evoked serotonin release on neurotransmission - relevant time-scales in the prefrontal cortex of mice to explore the effects of p-chloroamphetamine (pCA). Serotonin release is evoked via electrical stimulation of the medial forebrain bundle and monitored at carbon fiber microelectrodes. The dose response of pCA was determined in vivo and the suspected modes of action were tested using mathematical and pharmacological experiments. This fundamental mechanistic information about amphetamine and serotonin can be used to advance medical efforts to better treat debilitating mental disorders.

Keywords: Electrochemistry, Neurochemistry, Pharmaceutical, Voltammetry
Application Code: Neurochemistry
Methodology Code: Electrochemistry
Bio/Pharma Electrochemistry

Two-Electron Oxidation of Trolox in Phosphate Buffered Solutions

Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) is commonly used as a reference in the process of Trolox Equivalent Antioxidant Capacity (TEAC) assay, which was the most widely used method to evaluate antioxidant power of various compounds and biological materials. A considerable interest was focused on the redox and electrochemical characteristics of trolox in recent years. In this work, the electrochemical oxidation of trolox on glassy carbon (GC) electrodes was investigated by cyclic voltammetry (CV) in phosphate buffered solutions in the pH range of 5.0-11.93. Absorption and comproportionation are considered in the studies of the proposed mechanism for the first time. The absorption of trolox on the surface of GC electrode was confirmed by voltammetric technique and corresponding surface coverage value was calculated. According to the proposed EEC mechanism, two quasireversible one-electron transfers of deprotonated trolox formed phenoxyl radicals and phenoxonium ion. The generated carbocation decays following a pseudo-first order chemical reaction to produce its corresponding chromanone. Both the deprotonated trolox and phenoxonium ion undergo comproportionation. Trolox follows a similar proposed mechanism in different pH according to experimental results. In order to determine thermodynamic and kinetic parameters for the proposed reaction mechanism, digital simulation was used to fit the cyclic voltammograms.

Abstract Text

Keywords: Biofuels, Electrochemistry, Electrode Surfaces, Electrodes
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Electrochemistry
We have developed and applied state of the art nanoelectrochemical and mass spectrometric imaging methods to analyze the effects of intracellular zinc on cellular processes related to neurotransmission and learning and memory. In the central nervous system, a number of functions have been suggested for zinc. However, as important as zinc is, a mechanism of modulation of the response of a wide variety of ion channels and alteration of synaptic strength and plasticity to effect learning and memory by zinc has been difficult to establish. Exocytosis is the main mechanism of signal transduction and neuronal communication. Numerous studies in the last decades have suggested both learning and memory are somehow expressed through neurotransmitter release in nerve cells. Therefore, the mechanism by which zinc is involved in exocytosis and modulates neurotransmitter release or synaptic strength should provide insight into the function of zinc in learning and memory.

Single cell amperometry and intracellular impact electrochemical cytometry has been used to investigate the effect of micromolar zinc on exocytosis and vesicle storage in cultured pheochromocytoma (PC12) cells. With these two techniques, we have found that not only the vesicle content has been significantly changed after zinc treatment, but the dynamics of exocytotic release has also been affected. To get more insight into the mechanism of this process, we used TOF-SIMS analysis to investigate zinc distribution and localization in PC12 cells after zinc treatment. Two species of [ZnOH3]+ and [ZnO2H]+ were found to localize differently inside of the cells or on the cell membrane, which shows zinc might bind to proteins in both places to modulate the vesicle content and exocytosis.

REFERENCES

Keywords: Electrochemistry, Mass Spectrometry, Neural Network
Application Code: Neurochemistry
Methodology Code: Electrochemistry
Smart nanomaterials are an innovative alternative to support flexible and reprogrammable sample processing and tunable microscale bioseparations. The morphology of phospholipid nanogels is temperature dependent, rendering the viscosity thermally responsive. These material features are leveraged to integrate sample processing and complex separations in a single platform. By harnessing a smart material, the on-board fluid is programmed to concentrate, process, steer, and then separate targeted analyte. The reversible nanogel can be directed to different microfluidic channels using thermoelectric modules to spatially control the temperature in a microfluidic chip with a 2-second switching time. This is significant to chemical separations because generic microscale channels generate a sophisticated multifunctional device that can be patterned, used, erased, and reloaded repeatedly. These materials extend the range and precision of biomolecular size discrimination and support separation-based enzyme and lectin processing. The nanophase is key to this approach because it preserves the protein activity, supports catalytic processing using nanoliter volumes of enzymes and enhances electrophoretic separation of the products. The analyte resolution of biomolecules separated by these nanophases are exceptionally high, yielding separation efficiency up to 2 million theoretical plates. A critical advantage of self-assembled nanophases for efficient separations of biomolecules is the ease with which they are modified and then introduced, patterned, and replaced in the capillary. The applicability of this technology will be demonstrated with oligosaccharide and protein separations.

Keywords: Biotechnology, Capillary Electrophoresis
Application Code: Bioanalytical
Methodology Code: Capillary Electrophoresis
Capillary Electrophoresis/Electrochromatography-Mass Spectrometry for Pharmaceutical Analysis

CE/CEC-ESI-MS is a powerful technique for analysis and structure elucidation of active components in natural products. In recent years we focused our work on the development of new methodology of active components in natural products by CE/CEC-ESI-MS and structure elucidation of unknown components in Chinese medicine by IT-ESI-MS based on the fragmentation pathway of MSn.

This talk will introduce the advance in the analysis of active components in natural products by CE/CEC-ESI-MS in our group. It includes: sheathless nano-electrospray interface, novel column technology for CEC-MS, as well as CE/CEC-ESI-MS new methods for pharmaceutical analysis and elucidation of fragmentation pathway of active components by MSn.

Reference:

Acknowledgment
This work was supported by National Natural Science Foundation of China (21375101, 81573384, 90817103), the Natural Science Foundation of Hubei Province (No 2011CDB475).

Keywords: Capillary Electrophoresis, Mass Spectrometry, Pharmaceutical
Application Code: Drug Discovery
Methodology Code: Capillary Electrophoresis
Post-translational modifications (PTMs) are reversible, enzymatic modifications that contribute to protein diversity. Methylation can change chromatin structure and determine how genes are controlled and expressed. Lysine methyltransferases, KMTs, and lysine demethylases, KDMs, can change the lysine residue to be mono-, di-, or trimethylated. Depending on the location and level of modification, methylation is related to various neurological disorders and cancers; therefore, identification of this modification is important.

PTM detection is done via mass spectrometry; however, separation techniques are needed to enhance detection due to the low abundance of methylated residues compared to non-methylated ones. Liquid chromatography is commonly interfaced with MS but it is limited in resolution and lacks versatility of separation modes, based only on hydrophobicity. On the other hand, capillary electrophoresis (CE) provides advantages of high resolution, versatility of separation mode, and low reagent consumption. Antibodies are used to bind methylated lysine residues but they can be costly and time consuming to raise. Supramolecular hosts, such as cavitand and 4-sulfocalix-[4]-arene (CX4), are an attractive alternative because they are selective for different methylation levels and their structures can be modified to improve selectivity. We included supramolecular hosts in the running buffer of CE for highly effective separation of methylated and unmethylated guests. We determined the selectivity of CX4 against various methylation levels and discovered the host has the highest affinity for trimethylated fluorescein-derived guests. The host-assisted CE method is applied to separate methylated and unmethylated peptides and to detect the activity of enzymes involved in peptide methylation. Our work proves that a synthetic host is a useful tool for analysis of protein modification.

This research is funded by NSF Career #1057113.
This work aims to develop a capillary electrophoresis (CE) platform for investigating diverse protein-protein interactions (PPIs) using protein cross-linking. Although possessing many advantages for detecting, quantifying, and screening PPIs, CE is limited by the need to have separation conditions that maintain native protein interactions over the course of the separation which can be particularly challenging for weakly interacting proteins with high rates of dissociation. In protein cross-linking capillary electrophoresis (PXCE), proteins are incubated, cross-linked, and then separated by CE, facilitating method development by eliminating the need to maintain native interactions. Previously, PXCE methods for several nanomolar affinity PPIs using simple formaldehyde cross-linking procedures were reported. PXCE with formaldehyde cross-linking gives good quantitative results, e.g., a lysozyme-antibody interaction was found to have a $K_{d} = 24 \pm 3$ nM by PXCE and a $K_{d} = 17 \pm 2$ nM using isothermal calorimetry (ITC). Here we report that this approach appears to be limited to nanomolar interaction affinities. The PXCE method was adapted to access weakly interacting complexes by using rapid cross-linking reagents to evaluate micromolar affinity interactions including heat shock organizing protein (HOP) and heat shock protein 70 (Hsp70). The results were compared to accepted techniques: ITC, flow cytometry protein interaction assays, and affinity probe CE without cross-linking. These methods were sensitive to point mutations in the protein interaction sites and small molecule PPI inhibitors. The utility of PXCE will be further characterized for analyzing multicomponent complexes to allow for multiplexing and increased throughput for drug discovery.

Keywords: Bioanalytical, Capillary Electrophoresis, Drug Discovery, Protein

Application Code: Bioanalytical

Methodology Code: Capillary Electrophoresis
Single-cell analysis opens new possibilities to study cell-specific gene expression during embryonic development. Traditionally, mass spectrometry (MS) averages a large number of cells to obtain in-depth information on expressed protein. However, cell averaging provides little information on differences between individual cells. To detect cell-specific gene expression, we developed a capillary electrophoresis (CE) electrospray mass spectrometry approach for bottom-up proteomics. The instrument enabled an ~25-amol detection sensitivity and <20% RSD quantitative reproducibility for both technical and biological replicates. Next, we used single-cell CE-MS to compare gene translation for cells in the 16-to-128-cell frog embryo that have distinct tissue fate. To sample progressively smaller single cells in the embryos, we developed a microsampling approach that enable the collection of an ~20 nL portion of each identified cell, leaving the embryo essentially intact. Micro-collected proteins were digested and analyzed by the microanalytical CE-MS platform. This approach enabled the identification of ~250 different protein groups in a single cell, comparing well to the number of identified proteins from manually dissected cells from the embryo. At present, we are extending this approach to measure proteins in smaller cells at later stages of the developing embryo. We expect this technology to raise a potential to help understand how cell-specific gene expression underlies tissue and organ induction.

Keywords: Capillary Electrophoresis, Protein, Sampling, Small Samples
Application Code: Genomics, Proteomics and Other 'Omics
Methodology Code: Capillary Electrophoresis
The dysregulation of [beta]-endorphin, an endogenous opioid peptide found in the hypothalamus and pituitary gland, may be involved in the pathology of Autism Spectrum Disorders (ASD). [beta]-endorphin is known to exert regulatory effects on the secretion of oxytocin, which is a key hormone in the development and expression of social bonding behavior. To understand potential roles of [beta]-endorphin dysregulation in ASD, measurements of [beta]-endorphin and oxytocin secretions must be established in basal models with high spatial and temporal resolution. The ability to selectively measure these peptides from biological samples that contain many other peptides is a fundamental challenge for successful assay development of small peptide hormones. Immunoassays capitalize on the specific affinity of an antibody for its antigen to achieve high selectivity detection of peptide analytes. While current immunoassay methods are sensitive and selective, use of large sample volumes and long analysis times inhibit spatial and temporal resolution of the assays. Capillary electrophoresis (CE) immunoassays have proven advantages over other assay formats, including small sample volumes, reduced costs, quick analysis time, and automation capabilities. Here we describe the development of two new CE assays for measuring [beta]-endorphin and oxytocin. The first is based on conventional CE immunoassays, employing a [beta]-endorphin-specific antibody and an LED-induced fluorescence detection scheme. Both competitive and direct immunoassay formats were investigated. The second assay is a novel application of diagonal CE for the specific detection of oxytocin. Together these assays will enable high fidelity study of the regulatory relationships between [beta]-endorphin and oxytocin.
Metabolic characterization of single cells using mass spectrometry (MS) has opened new research potentials in cell and developmental biology. Using single-cell capillary electrophoresis electrospray ionization (CE-ESI)-MS, we recently uncovered cell heterogeneity across the three major developmental axes of the vertebrate embryo and identified metabolites that alter normal cell fates (Onjiko et al., PNAS 2015). However, microdissection of adjacent cells and/or smaller cells becomes increasingly laborious at later stages of development, thus making spatiotemporal investigations challenging. To overcome these challenges, here we developed and validated a microaspiration approach to enable local sampling of cellular contents from single cells within intact, developing embryos of the frog Xenopus laevis. Despite aspirating a miniscule, ~10 nL portion of the cellular cytoplasm, we were able to detect hundreds of chemical signals and identify more compounds than using microdissection. Closer inspection of the data revealed that microaspiration minimized interfering chemical signals from complex media. Next, we used this method to measure metabolite production in cell clones that form a common ancestor as the embryo develops from the 8- to the 32-cell stage. Statistical and multivariate analysis of the resulting complex data uncovered reorganization of the cellular metabolome as embryonic development spatiotemporally unfolded. This work addresses an analytical challenge in the direct spatiotemporal metabolic analysis of live, differentiating cells, raising broader adaptability to other cell types and cell/developmental models.

Keywords: Capillary Electrophoresis, Mass Spectrometry, Metabolomics, Metabonomics, Small Samples

Application Code: Bioanalytical

Methodology Code: Capillary Electrophoresis
Recently, our lab has developed a strategy for analyzing data from liquid chromatography-high resolution mass spectrometry (LC-HRMS) analyses using multivariate curve resolution-alternating least squares (MCR-ALS) to resolve pure analyte signals from the data giving rise to both chromatographic and spectral profiles, which can then be used for quantitation, pattern recognition, and/or compound identification. MCR-ALS analysis of LC-HRMS data in its raw form can easily overwhelm most desktop computers. The key to our strategy is the reduction in data complexity by removal of irrelevant signals arising from noise and background. Even so, some noise is still captured in resolved spectral signals, particularly for low-level analytes. In this talk, an improvement to this strategy involving a sparseness constraint will be described, taking advantage of the fact that mass spectra resulting from electrospray ionization should contain a minimum number of meaningful peaks per individual chemical species. This greatly facilitates the selection of relevant masses in our strategy and should enable further automation for this method.

This research is supported via a grant from NSF (CHE-1507332).

Keywords: Chemometrics, Data Analysis, Gas Chromatography/Mass Spectrometry, Liquid Chromatography/Mass Spectrometry, General Interest

Methodology Code: Chemometrics
Radiological mapping of potentially hazardous locations is one of the objectives of the U.S Environmental Protection Agency (USEPA) Airborne Spectral Photometric Environmental Collection Technology (ASPECT) project, providing first responders with a means of both managing and navigating areas of interest. The ASPECT project makes use of a unique fixed-wing aircraft outfitted with a gamma-ray spectrometer. The primary challenge in the development of a pattern recognition method capable of remotely identifying specific radioisotopes is obtaining gamma-ray spectra with a sufficient signal-to-noise ratio. Meeting this challenge requires optimization of both the spectrometer hardware and the associated data processing techniques. This research focuses on developing signal processing and pattern recognition methods that yield effective classification models for selected radioisotopes.

The design of classifiers for cesium-137 and cobalt-60 will be discussed as examples of the overall methodology. Specific topics to be addressed include (1) the assembly of training and test data based solely on laboratory spectra of the targeted radioisotopes combined with background spectra collected in the field and (2) the design of a confidence model that assigns a probability to the classification of each collected spectrum, enabling intelligent decision-making by first responders on the ground. In addition, a unique anomaly classifier based on the Compton region of the gamma-ray spectrum will be described. Rather than detect a specific radioisotope, this classifier is designed to detect spectra that exhibit signatures other than those associated with normal backgrounds. In conjunction with the radioisotope-specific classifiers, this methodology is presented as a comprehensive means of gamma-ray detection.

Keywords: Chemometrics, Detection, Environmental Analysis, Pattern Recognition
Application Code: Environmental
Methodology Code: Chemometrics
**Abstract Text**

Liquid chromatographic (LC)-MS approaches, which are based on ultrahigh pressure liquid chromatography (UPLC) systems typically feature a throughput of 4-5 minutes per sample which includes UPLC re-equilibration time. For high-throughput screening (HTS) such throughput is usually inadequate. Greater throughput has been achieved by combining MS with the RapidFire solid phase extraction (SPE)-based system to provide sample analysis speeds an order of magnitude faster with comparable data quality.

We have previously demonstrated this HTS RapidFire-SPE technology as an enabling technology for medicinal chemistry screening and hit-to-lead selection of small covalently binding acrylamide compounds to a target containing an active-site cysteine residue (1), with a total sample analysis time of 15s.

Here we present the same technology used for the analysis of intact (150 kDa) and reduced (25 kDa & 50 kDa) monoclonal antibodies in high throughput manner. There appears to be no detrimental ionization suppression effects when the light and heavy (glycosylated) chain co-elute from the SPE-cartridge. Accurate light and heavy chain molecular weights (±0.5 amu) can readily be determined, as can the glycosylation levels of the heavy chain.

Intact and reduced Antibody Drug Conjugates (ADCs) can also be readily analysed. The Drug-antibody-ratios (DAR) can also be accurately determined for the antibody-drug-candidates, on both the reduced and intact levels, and are highly consistent with DAR values calculated through traditional LC-MS analysis routines (18min LC-method).

This new HTS-SPE-MS acquisition and processing workflow has the potential to significantly increase sample throughput, data generation and analysis dramatically.


**Keywords:** Biopharmaceutical, Drug Discovery, Liquid Chromatography/Mass Spectroscopy, Time of Flight MS

**Application Code:** Drug Discovery

**Methodology Code:** Liquid Chromatography/Mass Spectrometry
I will discuss our recent efforts to develop a 3D liver culture platform capable of high-throughput screening of metabolism, cytochrome P450 induction and inhibition, as well as cellular health and hepatotoxicity. These cultures will mimic the complex cellular environments found in a liver acinus, and provide more representative data of in vivo response than the monolayer cultures currently used. We have developed a 96-zone format that supports paper-based liver cultures that are as easily prepared, maintained, and analyzed as current monolayer formats. These cultures utilize the wicking properties of the paper-based scaffolds to develop a gravity-driven “drip culture” in which compounds of interest are passed through the cultures, and the solutions collected for further analysis with validated methods for metabolic screening (e.g., quantitative LC-MS). We will specifically discuss how these cultures are being used to discover potential endocrine disrupting chemicals.

Keywords: Biomedical, Biotechnology, Lab-on-a-Chip/Microfluidics, Toxicology
Application Code: Drug Discovery
Methodology Code: Biospectroscopy
Electrochemical impedance spectroscopy is frequently used to characterize, optimize, and monitor ion-selective membranes. However, because of the relatively high resistance of ion-selective membranes, their impedance spectra often contain artifacts that can cause misinterpretation. While in the high-frequency range artifacts are often readily identifiable by the occurrence of inductive features or negative resistances, artifacts are easy to overlook in the low-frequency range, where telltale characteristics are typically missing. Some artifacts can be avoided by use of two-electrode cells, but this experimental design makes it hard to distinguish the impedance of the ion-selective membrane from that of the measuring electrodes. This work shows that experimental data can be analyzed accurately with the use of models that account for the capacitive leakage present in the reference channels of the impedance spectrometer. To test these models, valinomycin-doped K+-selective membranes were studied by electrochemical impedance spectroscopy with two-, three-, and four-electrode cells, using several measuring electrodes with low to high impedances. The models were found to correctly predict experimental data and provide an intuitive understanding of the cause of the impedance artifacts. This understanding can be applied to design electrochemical impedance spectroscopy experiments of ion-selective membranes with three- and four-electrode cells that minimize artifacts.
In military and commercial aerospace applications, carbon fiber epoxy composites (CFE) and high strength aluminum alloys (AA) are used primarily as structural materials due to their high strength to weight ratio. The service life of aircraft depends on the resistance of these structural materials to degradation and corrosion. In this work we aim to identify early stages of degradation observed in both carbon composites and aluminum alloys. Particularly the galvanic corrosion of aluminum when placed in contact with carbon composites. Electrochemical techniques such as impedance spectroscopy, cyclic voltammetry, linear sweep voltammetry, and galvanic corrosion measurements are used to quantify the level of corrosion experienced by CFE/AA coupled systems. CFE/AA systems were exposed to standard accelerated degradation testing (ASTM B117 and aqueous NaCl mist) to determine measurable corrosion levels over reasonable time scales.

The degradation of the CFE/AA system is hypothesized to rely on the formation and buildup of peroxide near the CFE fiber surfaces. Oxidation of carbon fibers leads to delamination of the epoxy matrix, both increasing the exposed surface area of the fibers and decreasing the structural stability of the composite. Increased surface area is also predicted to lead to greater galvanic corrosion in CFE/AA systems. This hypothesis was tested by exposing CFE composites aggressive environments and characterizing their electrochemical response.
Complementary electrochemical and photoelectrochemical techniques were utilized on CuWO$_4$ photoanodes prepared by a new atomic layer stack deposition-annealing (SDA) method to investigate the role of its surface states in water oxidation. Current density – voltage (JV) curves, electrochemical impedance spectroscopy (EIS), cyclic voltammogram (CV) surface studies and current transient measurements suggest that the charging of the CuWO$_4$ surface state is related to the turn-on of water oxidation. EIS data also show that the surface state is only observable under illumination, indicating that the surface state is not a permanent state in the electronic structure of CuWO$_4$ as proposed by previous literature. CV surface studies in non-aqueous electrolytes were further conducted to confirm that the surface state only exists when water oxidation occurs. Thus we argue that water oxidation on CuWO$_4$ takes place via a surface state, which is associated with the water oxidation intermediate.

Intensity-modulated photocurrent spectroscopy (IMPS) measurements were conducted to examine the rate constants of charge transfer and recombination processes at the CuWO$_4$ electrode surface. The hole collection efficiency was also extracted from the IMPS measurements. It is found that the main limitation of hole collection at CuWO$_4$ surface is the severe surface recombination in the low potential region.

The investigation of the surface state of CuWO$_4$ shines light on potential strategies to improve its hole collection efficiency, which should be focused on reducing surface recombination. The effect of various catalysts on CuWO$_4$ were also investigated by different electrochemical methods, and possible ways of passivating the surface recombination are proposed.

**Keywords:** Electrochemistry, Electrode Surfaces, Energy, Material Science

**Application Code:** Material Science

**Methodology Code:** Electrochemistry
Hg-based ultramicroelectrodes (UMEs) allow the extension of scanning electrochemical microscopy (SECM) investigations to ionic processes for the study of energy materials. However, existing Hg-based probes have either a sphere-cap or thin film geometry, both of which must be operated under dilute conditions and at rapid experimental timescales to avoid irreversible Hg loss due to saturation of the amalgam. This limits the chemical resolution of similar species, such as alkali ions, and hinders investigations in real-world environments. To facilitate ion-specific measurements in concentrated, multi-component solutions, we report a novel fabrication protocol for Hg disc-well UMEs, which maintain the chemical sensitivity of stripping reactions while also guarding against amalgam saturation. We compare the performance of Hg disc-wells and traditional sphere-caps in non-aqueous solutions containing multiple alkali ions and quantitatively describe the superior chemical resolution available to Hg disc-well UMEs. With support from Comsol simulations, we define limiting conditions for successfully resolving various ionic signals and provide a means of predicting the Hg disc-well dimensions required to resolve two particular species.

Following our interest in cyclic voltammetry probe approach surfaces (CV-PASs) based on alkali amalgamation and stripping, we demonstrate good fits of experimental and simulated Na(Hg) CV-PASs with an analytical model. By comparison, we find that the disc-well geometry facilitates smaller tip-substrate gaps than obtained with sphere-cap probes and boosts both spatial and temporal resolution in SECM studies. This is further demonstrated in SECM images extracted from the stripping signals of multiple alkali ions.

(2) Hui, J.; Burgess, M.; Zhang, J.; Rodríguez-López, J. ACS Nano 2016, 10 (4), 4248–4257.

Keywords: Electrochemistry, Energy, Microelectrode, Stripping Analysis
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Electrochemistry
Electrochemical Investigations of Energy Storage Materials

Alkali Ions Intercalation on Few Layer Graphene – Mechanistic Study and [i]In Situ[/i]
Electrochemical Imaging [i]via[/i] SECM

Bulk and surface electrochemical reactivity converge at ultrathin electrochemical interfaces like few-layer graphene (FLG). Despite the recent works regarding the use of graphene for charge storage, the mechanistic details of Li[\textsuperscript{+}] intercalation at FLG remain a mystery. Understanding this behavior, e.g. whether lithiation proceeds by a well-known staging type mechanism, will inform design parameters for the next generation of battery materials.

Here, we report a systematic study with controlled layer number FLG samples (1-10 layers) made by layer-by-layer transfer of bilayer graphene. The number of phase transitions between stages and their peak potentials were greatly affected by differences in the number of stacked graphene layers. The [i]in situ[/i] SEI growth and change in electronic activity at the FLG was monitored by Scanning Electrochemical Microscopy (SECM). Also, “non-traditional” Hg-based SECM probes were applied to image the local Li[\textsuperscript{+}] concentration change during intercalation.

The extreme thinness of FLG makes it a good candidate for studying the intercalation and co-intercalation mechanisms of alkali ions at fast scan rates. In particular, electrostatic interactions involved in the (de-)insertion behavior of alkali ions are difficult to observe traditional graphitic anodes, but are amplified at ultrathin FLG. We explore these interactions and their impact on Li[\textsuperscript{+}], as well as K[\textsuperscript{+}] and Na[\textsuperscript{+}] by modifying the exposed graphene and/or the buried graphene-substrate interface with a variety of electrostatic dopants. The model system of FLG provides unprecedented insights regarding ion intercalation mechanics, which will enable the rational design of battery materials with precisely controlled kinetics.


Keywords: Analysis, Electrochemistry, Energy, Material Science
Application Code: Material Science
Methodology Code: Electrochemistry
Solid electrolytes (SEs) for Li batteries are the ultimate solution for the electrode dissolution problems and safety hazards in liquid electrolyte (LE) systems. Sulfur based bulk SE attracts great interest due to their high ionic conductivity. Besides the Li conductivity, physical contact, material degradation and the kinetics of redox reaction at SE/electrode interface during battery processes are other key factors which dictate the battery performances. Currently the challenge of studying these interfaces in operando is assembling an air-tight spectro-electrochemical cell with good optical accessibility to the interface of interest. In this work, a cell is designed for the in situ Raman measurement at SE/Au interface during Li deposition and stripping processes. Three representative sulfur based SEs ([beta]-Li[sub]3[/sub]PS[sub]4[/sub], 70Li[sub]2[/sub]S-30P[sub]2[/sub]S[sub]5[/sub] glass ceramic (LPS-GS), Li[sub]10[/sub]GeP[sub]2[/sub]S[sub]12[/sub] (LGPS)) were investigated. Spectroscopic data shows that, in general, partially reversible structural interconversion occurs among PS[sub]4[/sub][sup]3−[/sup], P[sub]2[/sub]S[sub]6[/sub][sup]3−[/sup], P[sub]2[/sub]S[sub]7[/sub][sup]3−[/sup] and other unidentified P-S counter ions. Corresponding variations in cell impedance were also observed with these interfacial structural evolutions. Result reveals that in all solid Li battery systems, oxidation/reduction of Li[sup]+[/sup] occurs by breaking and reformation of the Li[sup]+[/sup] - Anion interactions in the SE materials, usually accompanied by the unexpected, partially irreversible structural evolution of the counter ions as the byproducts which are later accumulated at the electrode/electrolyte interfaces. Results not only builds the electrochemical fundamentals of the molecular details at solid-solid interface but also guide the choice of materials and the design of efficient interfaces.
Electrochemical double layers (EDL) in ionic liquid (IL) solvents demonstrate behaviors that are not easily explained by existing knowledge of aqueous counterparts. Theoretical work on ILs’ behavior near electrodes suggests the capacitance response should go through a maxima (bell-shape) or local minima surrounded by two maxima (camel-shape) at the potential-of-zero-charge (PZC). This is different from dilute aqueous salts which show a minima (U-shape) in capacitance near the PZC. Despite these and ongoing research efforts, debate surrounds the underlying causes and predictions of these behaviors in ILs. The present work uses large amplitude Fourier transformed alternating current (FT-ac) voltammetry to study the capacitance response as a function of the IL, electrode material, and ac frequency. The capacitance curves are generated from the first harmonic data, which is very sensitive to non-Faradaic charging currents. Data shows behavior analogous to dilute aqueous salts (U-shape) and high temperature molten salts (broad U-shape or flat) depending on the IL and electrode material. These results are in stark contrast to predictions from theory regarding ILs. The ac frequency displays an expected trend where lower capacitance is measured at higher ac frequencies. Taken together this capacitance data suggests the chemical nature of the IL EDL is similar to a dilute salt solution comprising an innermost layer, a transition layer, and terminating in the bulk. Experimental data is compared with current theoretical models to advance a working picture of the IL EDL.
Recent reports have revealed the importance of enzyme-mediated electrocatalysis in fine and specialty chemicals synthesis and for renewable energy applications. Enzymes offer high catalytic turnover per active site and selectivity. Moreover, enzymes can electrocatalyze many kinetically challenging reactions under ambient conditions in aqueous solutions. Our group focuses on studying the four-electron O2 reduction to water by bilirubin oxidase (BOD) on various nanostructure-modified electrodes. BOD is a multicopper enzyme that can perform O2 reduction at neutral pH with the least overpotential requirement compared to precious metal and other enzyme catalysts. However, existing challenges for utilizing BOD as a biocathode in fuel cells include low catalytic currents (affecting power output), electrode design issues, and poor stability of BOD films on electrodes. Hence, design of various nanomaterials for high-density immobilization of BOD with good stability and catalytic currents is an evolving research area. The inclusion of multiwalled carbon nanotubes (MWNT) into the BOD electrocatalytic system has shown to improve the catalytic current due to large surface area and conductive properties of MWNT facilitating high-density immobilization of catalytically active enzyme molecules. In this presentation, we show that BOD covalently attached to 1-pyrenebutyric acid pi-pi stacked on MWNT produces a greater electrocatalytic current than BOD covalently attached to chemically carboxylated MWNT. Moreover, we found that covalent BOD attachment provided higher catalytic currents than the corresponding noncovalent immobilization, possibly by allowing greater number of immobilized BOD molecules on the designed bioelectrodes. Thus, understanding subtle differences of nanostructure functionalization is significant in the design of enzyme bioelectrodes useful for electrocatalytic and fuel cell applications.
Application of mixed-metal oxides as active matrices is of particular importance to electrocatalytic oxidations of small organic molecules in low-temperature fuel cell technologies. The hydrous behavior, which favors proton mobility and affects overall reactivity, reflects not only the oxide’s chemical properties but also its texture and morphology. For example, during oxidation of ethanol (e.g. at PtRu), when rhodium nanoparticles have been dispersed in between tungsten oxide and zirconium oxide, significant current enhancements are observed. The result can be rationalized in terms of the formation of nanoreactors in which Rh induces splitting of C-C bonds in ethanol before the actual electrooxidation steps.

This electrode design was found to also promote the electrooxidation of As(III). The capability of various noble metals nanoparticles (Pt, Rh, Pd) to catalyze this process in acidic medium will be demonstrated. The recorded currents will be compared to those observed previously at the electrodes modified with a thin film of oxocyanoruthenate, perhaps the most potent system previously described for the electrocatalytic oxidation of arsenite. The reduction of As(V) is an even the more inert reaction. Network films of bimetallic (PtRu) nanoparticles (bare and derivatized) permit preconcentration of arsenic(V) species on their surfaces. The stripping step allows determination of arsenic at sub-micromolar levels.

Keywords: Chemically Modified Electrodes, Electrochemistry, Environmental Analysis, Nanotechnology
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Electrochemistry
We have developed an ultrasensitive gas-detection method based on the measurement of a differential capacitance of electrified ionic liquid (IL) electrode interfaces in the presence and absence of adsorbed gas molecules. The observed change of differential capacitance has a local maximum at a certain potential that is unique for each type of gas, and its amplitude is related to the concentration of the gas molecules. We establish and validate this gas-sensing method by characterizing SO2 detection at ppb levels with less than 1.8% signal from other interfering species (i.e., CO2, O2, NO2, NO, SO2, H2O, H2, and cyclohexane, tested at the same concentration as SO2). This study opens a new avenue of utilizing tunable electrified IL electrode interfaces for selective sensing of molecules with a kinetic size resolution of 0.1 Å.

Keywords: Adsorption, Electrochemistry, Environmental/Air, Sensors
Application Code: Environmental
Methodology Code: Electrochemistry
A novel fabricating method of microfluidic paper-based electrochemical sensor was developed. This microfluidic chip was fabricated by using an electronic digital plotter, which offers simple, rapid and precise fabrication process. The conductive pen containing the conductive ink (i.e. carbon nanotube and silver nanoparticle) in a ball pen was printed onto a photo paper substrate and the paper was cut according to our custom design with a blade in a machine. On this chip, the fluid was moved along the detection zone by using electrowetting power. The morphology of fabricated electrodes were characterized by scanning electron microscopy (SEM). For electrochemical characterization, such electrodes were investigated by cyclic voltammetry (CV) using a standard redox couple-ferri/ferrocyanide ([Fe(CN)6]3-/4-). SEM images verify the uniformity of the electrode surfaces, resulting in improved fabrication reproducibility. Furthermore, the CV results show the well-defined cyclic voltammogram with improved sensitivity of [Fe(CN)6]3-/4-. Ultimately, the fabricated electrodes were successfully applied for sensitive determination of bisphenol A-a toxic substance contaminated in foods and beverages. All of these results will be presented.

Keywords: Electrochemistry, Electrode Surfaces, Environmental, Lab-on-a-Chip/Microfluidics
Application Code: Environmental
Methodology Code: Electrochemistry
The concentration of hydrogen peroxide in various samples can be determined by using high performance anion exchange chromatography with pulsed amperometric detection (PAD). However, there have been reports that the sample matrices may dramatically affect the analytical results in the determination of hydrogen peroxide. Recently, we have performed studies to investigate the effects of various matrices such as water, sodium hydroxide and sodium chloride on the determination of hydrogen peroxide by using high performance anion exchange chromatography with PAD. The response, the retention time and the linear calibration were identified to vary from matrix to matrix. In order to eliminate the matrix effect, we developed a new PAD method for selectively detection of hydrogen peroxide. The new PAD methods overcome the matrix interference effect. We have also evaluated the new PAD method for analysis of hydrogen peroxide in different sample matrices. Excellent limits of detection and linear calibration results were obtained. The recovery was determined to be in the range of 95% to 104%.

Keywords: Bioanalytical, Detection, Electrochemistry, Ion Chromatography
Application Code: Environmental
Methodology Code: Electrochemistry
The ability to sense pH in aqueous solutions is fundamental to the study of chemical environments and is therefore prevalent in many industries. It is for this reason that the development of robust, reliable pH sensors has received significant interest from the scientific community for many years. The most prevalent pH sensor to date is the glass pH electrode, due to its high sensitivity to protons, large analysable pH range and relatively quick response time, as well as its commercial availability. It is however associated with several limitations including: being subject to ‘alkali errors’ and fragility due to the glass membrane, making in-situ pH detection problematic. Research has therefore focused on alternative methods of pH detection including fluorescence, pH-responsive polymers and other electrochemical approaches. pH dependent voltammetry of quinone groups on carbon electrodes such as glassy carbon and edge-plane pyrolytic graphite, has in recent years been used an alternative method of pH sensing. Boron doped diamond (BDD) as an electrode material has advantages over other carbon materials including: (i) high chemical and mechanical inertness; (ii) large solvent window; (iii) low background currents and (iv) biocompatibility.

Here, we detail the development of a voltammetric BDD pH electrode, which is formed by controllably adding sp\(^2\) regions, containing quinone functionalities, into high quality BDD, using laser micromachining. Importantly, the resulting pH sensor (Figure 1) exhibits the advantageous bulk physical properties of BDD, including low background currents, robustness, insensitivity to oxygen reduction, whilst accurately sensing pH over the investigated pH range 2-12.
The development of a sensor technology supporting the continuous, real-time measurement of specific molecules directly in the body could prove transformative in research and in medicine. In the short term, for example, such an advance would allow the in vivo concentrations of drugs and metabolites to be measured with high precision in subjects as they undergo their normal daily routine, improving our knowledge of physiology. On longer timescales, such an advance would facilitate “therapeutic drug monitoring,” in which dosing is personalized to the measured (rather than crudely or even indirectly estimated) metabolism of each individual patient. In response, we demonstrate here the ability of electrochemical aptamer-based sensors to support continuous, multi-hour measurements directly in living subjects. Specifically, we have used aptamer-based sensors to perform real-time measurements of drugs in the bloodstream of even awake, ambulatory rats, achieving precise molecular measurements at clinically relevant detection limits and high temporal resolution, attributes suggesting the approach could provide an important new window into the study of physiology and pharmacokinetics.

Keywords: Biosensors, Drugs, Electrochemistry, Medical
Application Code: Bioanalytical
Methodology Code: Electrochemistry
Background-subtracted fast-scan cyclic voltammetry combined with principle component analysis (PCA) has emerged as a powerful analytical technique for monitoring sub-second molecular fluctuations in tissue. Despite increased utilization, work to advance quantification of the electrode response has been limited. PCA calibration enables identities and concentrations of analytes to be assigned using analyte-specific voltammograms and scaling factors which fluctuate \textit{in vivo}, possibly resulting from impedance changes. Unaccounted for, this error leads to over or under prediction of analyte concentrations. Variable electrode performance and microenvironment contributions render calibration necessary for accurate quantification. However, protocols such as those for permanently implanted electrodes make conventional post-calibration difficult, and the use of a standard training set and scaling factor may not be appropriate. Previous research utilized total background current to predict electrode-specific scaling factors \textit{in situ}. In this work, the oxidation potential of surface-quinones in the background (Ep,QH) is shown to predict the oxidation potential for dopamine (Ep,DA) \textit{in situ}. We have developed an approach employing model circuits to simulate impedance changes \textit{in vivo}. High correlation of Ep,QH and Ep,DA ($R^2 = 0.94$) to the impedance of the preparation enabled these relationships to be exploited for controlled, \textit{in vitro} studies. To validate this approach, electrodes were permanently implanted \textit{in vivo} to monitor how both Ep values shift over multiple weeks. Utilizing our methodology, Ep,QH served as a reliable predictor for Ep,DA, leading to appropriate training set selection and a more accurate calibration. This advance will lead to more reliable complex measurements to investigate pharmacological and behavioral manipulation.
In-Vivo and Neuro Electrochemistry

Comparison of Acute and Chronic Electrodes for Fast-Scan Cyclic Voltammetry in Awake Animals

Fast-scan cyclic voltammetry (FSCV) has been used for over twenty years to study rapid neurotransmission in awake and behaving animals. These experiments have been historically carried out with fresh carbon-fiber microelectrodes (CFMEs) inserted into the brain through micromanipulators and guide cannulas. However, due to insertion damage and electrode fragility, only a few separate measurement sessions are feasible in a single animal. Moreover, it is not practical to make measurements in the same recording location over multiple days. With the aim of overcoming these limitations, chronically implanted CFMEs have recently been developed for longitudinal FSCV studies of neurotransmitter release. These electrodes are secured in particular brain locations for weeks to months, and have thus been utilized to make measurements of dopamine release over extended time scales that have not been possible with acute implantations.

While there are many advantages to chronic CFMEs, there are key considerations that must be made as this technology is developed. Differences in experimental design, implantation tactics, electrode stability, and data analysis could create differences between data collected with acute and chronic electrodes. Here we investigate the differences between these two experimental approaches, and suggest how FSCV data collected with these different preparations can be reconciled. Moreover, our goal is to highlight critical areas for future improvement and innovation as the use of chronic CFMEs continues to grow.

Keywords: Calibration, Electrochemistry, Microelectrode, Neurochemistry
Application Code: Neurochemistry
Methodology Code: Electrochemistry
In-Vivo and Neuro Electrochemistry

PEDOT-Based Electrode Coatings for the High Sensitivity Detection of Dopamine In Vivo

Neurotransmitters, such as dopamine (DA), are responsible for the regulation of a variety of vital life functions. Furthermore, dysfunction in neurotransmitter signaling pathways has been implicated in the onset of various neurological disorders. In vivo detection of neurotransmitters poses an interesting analytical challenge due to the low concentration and high speed of physiological signaling. Fast scan cyclic voltammetry (FSCV) has been performed at carbon fiber microelectrodes (CFEs) for the in vivo detection of neurotransmitters for decades, but the technique is limited by issues resulting from moderate sensitivity and poor sensor longevity. We have shown that electrodeposition of poly(3,4-ethylene dioxythiophene) (PEDOT) containing coatings onto carbon electrode surfaces increases the sensitivity and lowers the limit of detection for DA. PEDOT/graphene oxide coated CFEs exhibit an 880% increase in DA detection sensitivity using FSCV, a 50% decrease in DA lower limit of detection and minimally altered electron transfer kinetics. Furthermore, these electrodes are capable of detecting electrically evoked real-time DA transient activity in vivo. PEDOT/carbon nanotube coated CFEs exhibit a 4736% sensitivity increase for DA detection using differential pulse voltammetry and exhibit 34% DA retention when removed from a 10 µM DA solution. This retention has the effect of pre-concentrating analyte onto the electrode surface and increasing electrochemical signal. Overall, PEDOT-based coatings significantly improve DA detection capabilities both in vitro and in vivo and is expected to considerably improve our understanding of the DA function in vivo.

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Keywords: Electrochemistry, Electrode Surfaces, Neurochemistry, Polymers & Plastics
Application Code: Neurochemistry
Methodology Code: Electrochemistry
Pain management is one of the oldest problems in medicine. Unfortunately, a precise understanding of how opioid neuropeptides underlie pain (and pleasure) is lacking largely due to an inability to monitor these endogenous species \textit{in vivo}. We have developed a voltammetric approach for the real time quantitation of tyrosine-containing opioid neuropeptides. Fast scan cyclic voltammetry is an established electroanalytical technique in the field of neuroscience, used primarily to study the dopaminergic system. When coupled to carbon-fiber microelectrodes, this approach offers selective real-time measurements that provide unparalleled temporal and spatial resolution. This work characterizes innovative modification of the standard voltammetric waveform to allow for the rapid, sensitive and selective detection of methionine-enkephalin (m-ENK), an endogenous opioid neuropeptide. We have systematically characterized and optimized application rate, holding potential, acclivity potential, scan rate and accumulation potential. These modifications have allowed us to overcome a multitude of challenges associated with the quantitation of m-ENK \textit{in vivo}. Enabling the real time detection of m-ENK fluctuations \textit{in vivo} will further the understanding of the role of m-ENK in multiple biological pathways.
In-Vivo and Neuro Electrochemistry

Electroanalytical Measurements of Tyrosine-Containing Neuropeptides: Chasing the Enkephalins

Opioid neuropeptides regulate a broad spectrum of biological functions and are heavily implicated in pain management and in hedonic behaviors associated with addiction. Opioid neuropeptides modulate the mesolimbic and nigrostriatal dopamine (DA) circuits and intact opioid signaling pathways are required for key aspects of cocaine abuse. However, the precise mechanisms that underlie opioid modulation of DA systems remain ambiguous. Although several methods exist for monitoring DA fluctuations, few tools are available for selectively monitoring dynamic fluctuations of endogenous opioid neuropeptides. This work addresses the issues involved with monitoring endogenous opioid neuropeptides by characterizing and employing an innovative electrochemical approach to monitor sub-second fluctuations of the tyrosine-containing opioid neuropeptides methionine-enkephalin (M-ENK). By combining multiple scan rate voltammetry with constant-potential amperometry, we have optimized an electroanalytical technique for measuring M-ENK fluctuations. The waveform development described in this work has leveraged the knowledge gained from years of utilizing electrochemistry to monitor dopamine, and adapted it to M-ENK detection. Given that opioid signaling has been implicated in mechanisms accompanying robust increases in extracellular DA elicited by L-DOPA or cocaine, our study investigates cocaine-induced effects on putative M-ENK fluctuations in the striatum of an L-DOPA treated rat.

Keywords: Bioanalytical, Electrochemistry, Neurochemistry, Peptides

Application Code: Neurochemistry

Methodology Code: Electrochemistry
In order to perform analytical measurements in the brain, it is important to understand the local tissue environment. The formation factor (fF) is a dimensionless number that measures the degree to which a porous medium impedes transport and is dependent on the porosity and tortuosity. Currently, there is no method that can monitor local porous properties in vivo with high spatial resolution without the method itself perturbing the system. We have developed an analyte-free electrochemical technique that uses micro-conductivity measurements to determine the fF of porous media. Excitation of a 7-μm carbon fiber microelectrode in the medium (free or porous) with an AC current (10-100kHz) gives a frequency-dependent AC potential that depends on the electrical resistance of the solution, which directly depends on fF. We developed a simple circuit model and demonstrated that there is good agreement between model predictions and experimental conductivities of different concentrations of KCl. We demonstrated the applicability of our technique by measuring the fF of a brain surrogate: a bed of 0.6% agarose gel (in 0.9% NaCl). By comparing the AC potential in the gel to that of the free solution, we calculated the fF to be 0.20 ± 0.02. The reported porosity for this composition of agarose gel is 0.3\textsuperscript{1}; we thus estimated the tortuosity of the agarose bed to be 1.25 ± 0.08. We recently miniaturized our circuit so that it can be used for in vivo experiments. We have shown that we can reproducibly target and measure the conductivity of cerebrospinal fluid in the lateral ventricle.

References:

Keywords: Bioanalytical, Electrochemistry, Microelectrode, Neurochemistry

Abstract Text

In order to perform analytical measurements in the brain, it is important to understand the local tissue environment. The formation factor (fF) is a dimensionless number that measures the degree to which a porous medium impedes transport and is dependent on the porosity and tortuosity. Currently, there is no method that can monitor local porous properties in vivo with high spatial resolution without the method itself perturbing the system. We have developed an analyte-free electrochemical technique that uses micro-conductivity measurements to determine the fF of porous media. Excitation of a 7-μm carbon fiber microelectrode in the medium (free or porous) with an AC current (10-100kHz) gives a frequency-dependent AC potential that depends on the electrical resistance of the solution, which directly depends on fF. We developed a simple circuit model and demonstrated that there is good agreement between model predictions and experimental conductivities of different concentrations of KCl. We demonstrated the applicability of our technique by measuring the fF of a brain surrogate: a bed of 0.6% agarose gel (in 0.9% NaCl). By comparing the AC potential in the gel to that of the free solution, we calculated the fF to be 0.20 ± 0.02. The reported porosity for this composition of agarose gel is 0.3\textsuperscript{1}; we thus estimated the tortuosity of the agarose bed to be 1.25 ± 0.08. We recently miniaturized our circuit so that it can be used for in vivo experiments. We have shown that we can reproducibly target and measure the conductivity of cerebrospinal fluid in the lateral ventricle.

References:
Adenosine is an endogenous nucleoside in many important biochemical processes throughout the body. One way adenosine signals is by acting as a vasodilator and regulating cerebral blood flow during normal physiologic alterations in brain function. The correlation of spontaneous, transient adenosine and oxygen has been characterized on subsecond time scale. However, this mode of adenosine and oxygen changes have not been studied under ischemia-reperfusion (I-R) injury. In the present study, we use fast-scan cyclic voltammetry (FSCV) at carbon-fiber microelectrode to determine changes in concentration and frequency of correlated adenosine and oxygen during I-R injury. This technique allows simultaneous measurement of adenosine and oxygen release. Our results demonstrated that the number of both adenosine and oxygen transient events significantly increased during I-R injury but the concentration of adenosine and oxygen did not significantly change. The A$_{2a}$ antagonist, SCH442416, did not significantly affect the concentration or number of simultaneous adenosine and oxygen release under I-R injury. These results are surprising since the supply of blood, which carries oxygen to cerebral blood flow, is not stopped during ischemic injury. Thus, adenosine is able to increase oxygen transiently, even during a period of ischemia. This method demonstrates that adenosine and oxygen are correlated and that FSCV is useful for monitoring the neuromodulatory effects of adenosine.
In general there are two ways to stop microbes from infecting or deteriorating materials—disinfection and antimicrobial surfaces. The first is usually realized by disinfectants, which are a considerable environmental pollution problem. Antimicrobial and/or antifungal surfaces are usually designed by the penetration of materials with biocides that are released into the surroundings where upon microbes are killed.

Control with current fungicides, both systemic and contact fungicide options, have not been totally effective to control Cercospora leafspot in sugarbeets. We have been evaluating the effectiveness of a covalently bound antimicrobial/antifungal organosilane formulations against Cercospora leafspot fungus.
Chemical Methods

Comparison of Two Strategies for the Synthesis of Fluorescent Carbon Nanoparticles

Fluorescent carbon nanodots (C-dots) are carbon nanoparticles with typical sizes below 10 nm.[1] These nanomaterials are reported to display excellent chemical, physical, and photochemical properties (e.g., excitation wavelength dependent photoluminescence behavior, chemical inertness, low cytotoxicity, and good biocompatibility). Such characteristics has attracted considerable attention of many researchers because of the C-dots potential for a wide range of applications.[2]

Driven by the requirements of diverse applications, many efforts in recent years have been devoted to produce C-dots with desirable physical and spectral properties. Advances in this area are emerging frequently with a number of synthetic strategies (e.g., electrochemical oxidation, hydrothermal treatment, laser ablation, plasma treatment, and microwave-assisted heating) reported.[1] However, it is important to note that C-dots produced by different synthetic methods showed significant differences in size and spectral properties, even though the starting materials used for synthesis may have been the same. In order to produce C-dots with a specific set of characteristics for a particular application, it is necessary to investigate whether different synthetic methods generate different fluorescent species in the as-synthesized fluorescent products.

In this work, we aim to compare two of the most commonly reported approaches to synthesize C-dots, namely the hydrothermal treatment and microwave-assisted heating of simple molecular precursors. Citric acid (CA) and 1,2-ethylenediamine (EDA) were used as the C-dots precursors. The as-synthesized fluorescent products were characterized by various characterization methods, including transmission electron microscopy (TEM), infrared (IR) spectroscopy, UV-vis absorption and photoluminescence spectroscopy. In addition, the as-prepared C-dots products were analyzed by capillary electrophoresis (CE) coupled with laser induced fluorescence (LIF) detection. The fluorescent products derived from the two approaches showed much difference in their chemical structural and optical properties. The CE separations showed variation in the complexity of the fluorescent products derived from the two approaches.

References

Keywords: Capillary Electrophoresis, Nanotechnology, Surface Analysis, UV-VIS Absorbance/Luminescence
Application Code: Nanotechnology
Methodology Code: Chemical Methods
A New Method for the Analysis of Total Nitrogen in Aqueous Samples

A new, recently validated, ASTM method measures total nitrogen, and TKN by calculation, in water by high temperature catalytic combustion with chemiluminescence detection. The method was multiple laboratory validated with nine matrices. This poster presents a description of the method and summarizes the validation data.

Keywords: Environmental Analysis, Environmental/Water, Wet Chemical Methods

Application Code: Environmental
Methodology Code: Chemical Methods
Soybeans and beans are highly consumed and are known for having health benefits due to their antioxidant properties. There are several types of soybeans and beans, differing in color, shape and size. Colorful grains are known to have high antioxidant activities, and these compounds may have different solubility. The objective of this study was to evaluate the total phenolic contents (TPC) and the antioxidant activity of three cultivars of [i]Phaseolus vulgaris L.[/i] (black, purple, light-colored bean) and three cultivars of [i]Glycine max[/i] (black, green, yellow soybean) of the water, methanol and hexane soluble fractions of each legume. The Folin-Ciocalteu and the DPPH radical scavenging methods were used to determine TPC and the antioxidant activity of all fractions. The water fraction showed significantly (p<0.05) highest amount of TPC (1,12 ± 0,07 to 2,34 ± 0,01 mg gallic acid equivalent GAE g⁻¹ fresh matter; FM) and antioxidant activity (0,46 ± 0,03 to 2,13 ± 0,10 mg Trolox equivalent g⁻¹ FM) than the other fractions. The water fraction of black soybean (BS) exhibited significantly greatest phenolic content followed by yellow soybean (YS), green soybean (GS), purple bean (PB), black bean (BB) and light-colored bean (LB) (2,34; 1,91; 1,87; 1,70; 1,61; 1,12 mgGAE g⁻¹ FM, respectively). The antioxidant activity did not follow the same pattern (BB>BS>PB>LB>YS>GS), but still the dark-colored legumes presented significantly higher antioxidant activity (BB=2,13; BS=1,76; PB=1,46mg Trolox equivalent g⁻¹ FM). These results indicate that the water soluble fraction, especially for the dark-colored legumes, have the best potential for health promotion.
The olive oil is recognized for its beneficial effects on health. These benefits have been associated with its well-balanced fatty acid composition, of which oleic acid is the main component and to the presence of minor biomolecules, such as vitamins, carotenoids, tocopherols and phenolic compounds. Besides, these compounds have incidence not only on nutritional characters, but on sensory properties as well. These properties include bitterness, pungency, astringency and flavor. For this reason is important have a methodology that allows its determination quickly and easily.

Nowadays, continuing with the trends of reducing time and costs, QuEChERS (an acronym for Quick, Easy, Cheap, Effective, Rugged and Safe) has revolutionized the determination of different analytes in food matrices. QuEChERS, initially established by Anastassiades et al., has grown in popularity among pesticide residue determination, mainly for fruit and vegetable samples. This technique has been used for other analytes, such as phenolic compounds, however, at the moment; QuEChERS has not been reported for determination of phenolic compounds in olive oil samples.

For this reason in this work a QuEChERS using acetonitrile acidified (acetic acid 1% v/v), 2 g of MgSO4 and 0.5 g of NaCl for extraction and C18, CaCl2 and PSA (20 mg each) as dispersant during d-SPE procedure, was development. This methodology was employed for achieve appropriate extracts for to be injected in HPLC using a column of core-shell C18 for separation and consequent spectrophotometric determination. In this way LOD, LOQ, RSD and others analytical figures of merit, allowed the determination of 22 minor phenolic compounds (i.e. gallic, syringic, p-coumaric, ferulic, sinapic, kaempherol and vanillic acid, 3-hydroxytyrosol, tyrosol, quercetin 3-glucoside, rutin, kaempherol 3-glucoside, pinoresinol, 4-hydroxyphenylacetic, 2,5-dihydroxybenzoic, oleuropein, luteolin and apigenin) present in EVOO elaborated in Argentina.

Keywords: Chromatography, Food Identification, HPLC Columns, Lipids
Application Code: Food Identification
Methodology Code: Chemical Methods
As superparamagnetic cobalt oxide nanoparticles are attractive candidates for magnetic resonance imaging, magnetic field-assisted drug delivery and magnetothermal therapy it is important to obtain monodispersed particles with good magnetic characteristics, which mostly depends on nanoparticles size and stabilization shell [1,2]. This study is focused on the synthesis of cobalt ferrite (CoFe$_2$O$_4$) nanoparticles by high temperature solvothermic synthesis seeking to better control their size, purity and magnetic properties. In this way, solvothermic syntheses were performed using benzyl ether, Co(II) acetylacetone and Fe(III) acetylacetone. Different additives, such as dodecylamine and oleic acid, were tested seeking to obtain pure phase and monodisperse nanoparticles in average size of 6 nm at a good yield. Scanning electron microscopy (SEM), X-ray diffraction, Mössbauer spectroscopy and magnetic measurements were employed in this study.

It was determined that high temperature solvothermic synthesis is good technique to obtain 5-6 nm-sized, non-aggregated magnetic particles with low size distribution. As well, it was determined that it is possible to form those particles by adding oleic acid and removing water by evaporating from the synthesis solution (Fig.1).

References


Keywords: Chemical, Nanotechnology, Particle Size and Distribution, X-ray Diffraction
Application Code: Nanotechnology
Methodology Code: Chemical Methods
Fluorescence and Luminescence

Case Study of Nanoparticle-Protein Interaction: Conformation and Activity of Catalase can be Changed by Nanoparticles

Along with emerging application of nanoparticles in biomedicine and health care, it has become more and more important to undercover that myth of nanoparticles-protein interactions, which would dominate the biological identity and physiological response of nanoparticles. Previously, we have established one high throughput method using fluorescamine to label primary amine on the surface of protein, to study the conformational change or blockage caused by binding to nanoparticles. Here, we further studied the effect of conformational change caused by nanoparticles on protein functionality. Catalase was chosen for study because it shown larger fluorescence change in previous screening, as well as the truth that catalase is an important ROS (reactive oxygen species) scavenger in living organisms exposed to oxygen. Our preliminary data shown the activity of catalase to decomposite of hydrogen peroxide to oxygen was compromised after incubated with both polystyrene and silica nanoparticles, which might be caused by the conformational change. But the activity can be restored if some other proteins were co-incubated with nanoparticles, which indicated those proteins can compete with catalase to bind nanoparticles and thus protect its conformation. The restore effect can be different for different proteins, which might be caused by the difference of binding affinity toward nanoparticles. Furthermore, we used limited trypsin digestion to confirm the conformational change induced by nanoparticles and protection effect of other proteins.

Keywords: Bioanalytical, Fluorescence, Nanotechnology, Protein
Application Code: Bioanalytical
Methodology Code: Fluorescence/Luminescence
Heavy metal contamination is a major threat to human health. Studies have shown that microorganisms including fresh water algae are sensitive to high concentrations of heavy metals presence in water bodies. Impact of heavy metals on fresh water algae can be monitored via their chlorophyll content. Characteristic chlorophyll fluorescence provides a unique opportunity to develop non-staining thus non-invasive flow cytometric technique for algal bioassays. The current study investigates short-term toxicity effect of three common heavy metals found in water sources. Here we present the toxicity impact of Uranium (U), Arsenic (As), and Lead (Pb) on freshwater algae, [i] Chlorella [/i] genus. The toxicological effect of these metals on algal cell size, shape/granularity, and chlorophyll a were investigated over 72 hour period. Exponentially growing algae were exposed to varying concentrations of these three heavy metals. Concentrations of heavy metals were selected based on Environmental Protection Agency (EPA)'s Maximum Contaminant Level (MCL) allowed in drinking water. We have determined that exposure to U has significant impact on algal cell size, growth, and autofluorescence. Flow cytometry studies further reveal that exposure to As (V) has significant impact on [i] Chlorella [/i] while exposure to As (III) and Pb has comparatively lesser effects. The varying degree of sensitivity of [i] Chlorella [/i] towards these metals can be utilized for better understanding of environmental impact of heavy metals.

Funding for this work was provided through Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant number P20GM103451.
Fluorescence and Luminescence

Spyrolactum Capped Cyanine Dyes for Designing NIR Probes to Target Multiple Metal Ions

Conjugated cyanine (CY) dyes are well known for fluorescence imaging applications in biological systems due to their near-infrared (NIR) absorption and emission. However, designing of metal ion sensors based on CY moiety is challenging, the major problem in these area is quenching the fluorescence of the CY dye. Herein, we have reported a new, simple and effective strategy to synthesize CY based NIR probes which can be tuned for targeting different metal ions in aqueous solution. The new functional CY dyes are locked in a spirolactum capped non-conjugated form and thus most of the fluorescence is quenched. The spirolactum ring over the CY moiety breaks only in presence of specific metal ions and the selectivity towards metal ions can be tuned by modifying the binding site attached to the spirolactum moiety. The breaking of spirolactum ring produces the highly conjugated CY unit with a vivid change in absorbance and fluorescence spectra. In this report we have shown a pair of CY based NIR fluorophore [b]Cy-NHS[/b] and [b]Cy-PYR[/b]. In case of [b]Cy-NHS[/b] we have coupled the process of spirolactum ring opening with the stoichiometric and irreversible Hg[sup]2+[/sup]-induced conversion of thiosemicarbazides to 1,3,4-oxadiazoles. The CY derivative [b]Cy-NHS[/b] is perfect for this application since the thiosemicarbazide moiety of [b]Cy-NHS[/b] can be easily liberated to form the oxadiazole in presence of Hg[sup]2+[/sup] ions, inducing the ring opening of the spirolactum unit. In case of [b]Cy-PYR[/b] we have designed a metal binding site containing one N- atom from a pyridine moiety, one N- atom from an imine linkage and one O- atom from a ketone group as the electron donors. This assembly of three perfectly spaced electron donating atoms is well known for binding transition metal ions like Cu[sup]2+[/sup]/Zn[sup]2+[/sup]/Cd[sup]2+[/sup] etc. In fact the chemosensor [b]Cy-PYR[/b] is found to be selective towards Zn[sup]2+[/sup]/Cd[sup]2+[/sup] metal ions, the binding of Zn[sup]2+[/sup]/Cd[sup]2+[/sup] metal ions triggered the opening of spirolactum ring of the CY derivative, which was easily detected through switch ON response in absorbance and fluorescence spectra. For theoretical understanding of the study, a detailed DFT calculation of the chemosensors in absence and presence of metal ions has also been performed. We expect that this new technique can be applied for developing NIR sensors for various environmentally and biologically important metal ions.

Keywords: Fluorescence, Mercury, Near Infrared, Sensors
Application Code: Environmental
Methodology Code: Fluorescence/Luminescence
In our work, we proposed a novel approach to quantification of relatively small amounts of water present in low polarity, aprotic solvents using fluorimetry. This approach takes advantage of interaction of pair of the fluorescent receptor (4-methylumbelliferone) and optical transducer – non-fluorescent dye (Sudan 1), resulting in improved spectral selectivity of water quantification. In the presence of water in the aprotic solvent, the interaction of receptor and transducer lead to formation of a new emission peak of intensity related to the water contents in the system. The nature of interactions between molecules leading to a new peak formation was investigated using crystallographic approach as well as fluorescence lifetime measurements. The selectivity of the method has been improved compared to the system present in previous work [1] by shifting fluorescence wavelength.

Post-transitional modifications (PTMs) are playing important roles in regulation of gene transcription, strongly impacting cellular process, including differentiation, proliferation, apoptosis and epigenetics in both physiological and pathophysiological conditions. They also respond to different stimulations, leading to alterations in biological processes and development of pathological conditions. Monitoring PTM changes in cells is essential in epigenetics and systems biology for better understanding of the regulation mechanisms of cellular processes, and the enzymes that catalyze PTM reactions are potential targets for the treatment of diseases associated with epigenetic disruption. Synthetic receptors are an inviting solution to this problem, and there has been some elegant work recently published on the use of designed host molecules that bind to protein PTMs. A far more attractive strategy would be to employ a displacement sensor that works with common, variable fluorophores as detectors, but that requires a novel type of indicating mechanism and a host: guest recognition system. In this research, a rhodamine and cavitand based indicator displacement sensing system has been created for the detection of trimethylated peptides and determination of histone demethylase activity. The combination of high selectivity and sensitivity make this an idea method for enzyme inhibitor screening.

Keywords: Bioanalytical, Biosensors, Sensors
Application Code: High-Throughput Chemical Analysis
Methodology Code: Fluorescence/Luminescence
Session Title: Fluorescence and Luminescence

Abstract Title: Hybrid Micro/Nanogel Particles for Cellular ROS Measurement

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Abstract Text
Microgel and nanogel are particles with a chemically crosslinked polymer network. Over the past two decades, micro/nanogel particles have attracted much attention due to their capacity to be potential drug-delivery and chemical separation system. They are also widely adopted as materials for biosensing, optical device and micromechanical. In this research, copper sulfide nanocrystal enclosed microgel with three different size were fabricated first, and the performance of the microgel as a label for chemiluminescence detection has been shown to be superior comparing with HRP (horseradish peroxidase) label. Briefly, microgel particle was fabricated using a one pot synthesis method with NIPAM-co-acrylic acid-co-allylamine. Copper sulfide nanocrystals were fabricated in situ by coordinating copper ions with carboxyl group inside the microgel first and adding the sodium sulfide later. Upon adding of H2O2 and ABEI (N-(4-aminobutyl)-N-ethylisoluminol), strong chemiluminescence can be generated. With this design, microgel could serve as a micro-reactor to form numerous CuS nanocrystals, which increase the sensitivity towards ABEI based chemiluminescence assay. IgG based ELISA assay has been used as a model to verify the working principle. This method can be further used for other detection with simply changing the antibody pair. The future study will be using this setup to do exosome measurement coupling with a size separation filter.

Keywords: Luminescence, Nanotechnology

Application Code: Bioanalytical

Methodology Code: Fluorescence/Luminescence
Boron dipyrromethene (BODIPY) dyes are well-known to be highly fluorescent, very stable, having narrow emission bandwidths and amenable to structure modification. We have developed two new highly fluorescent probes based on boron-dipyrromethene functionalized with different groups. Boronic acid functionalized boron dipyrromethene (BABDP) was studied as a new fluorescent probe for the detection of catechols and catecholamines. The fluorescence of BABDP can be strongly quenched by dopamine due to the photoinduced electron transfer from phenyl borate ester to the BODIPY core. The probe was applied for the detection of dopamine from $10^{-8}$ to $10^{-2}$ M in homogeneous assay, and the selectivity toward dopamine was greatly improved by using hydrophobic polymer films containing BABDP and cation exchanger. Piperidine functionalized boron–dipyrromethene derivative with phenylamino (NHBDP) was developed as pH indicators. The piperidine units on BODIPY core not only provided the excitation and emission at visible wavelength, but also changed the energy level of excited fluorophore inducing an oxidative PET process upon deprotonation. The probe was further applied to prepare fluorescence turn-on cation selective optodes based on ion-exchange sensing mechanism, which was fabricated to measure Pb2+ with excellent sensitivity and selectivity.

**Keywords**: Fluorescence, Sensors

**Application Code**: Bioanalytical

**Methodology Code**: Fluorescence/Luminescence
In previous work, other research groups have demonstrated that bacteria and viruses can be separated by capillary. This achievement is significant because it shows that microbes have distinct electrophoretic mobilities that can be used to aid in their identification. However, capillary electrophoresis separations of bacteria is challenging because they often aggregate, which alters their electrophoretic mobility. To overcome this challenge we are developing a microfluidic device for rapid and iterative electrophoretic mobility measurements of single particles, including bacteria and viruses. This approach allows for discrimination of particle aggregates, making the identification process more robust. To increase the selectivity of the process for the identification of bacteria, an immunoassay is integrated directly into the process. We have successfully measured the electrophoretic mobility of individual particles and have shown that the precision and accuracy are equivalent to those obtainable by capillary electrophoresis. Current work is focusing on the measurement of the electrophoretic mobility of native E. coli with selective recognition of E. coli by binding of individual cells to fluorescent polystyrene probe particles decorated with anti-E. coli. We anticipate that by combining bead based immunoassays and the electrophoretic mobility characterization of individual cells, that the false-positives produced by non-specific binding with the antibody can be greatly reduced.

Keywords: Bioanalytical, Electrophoresis, Immunoassay
Application Code: Bioanalytical
Methodology Code: Fluorescence/Luminescence
A UV-Visible spectrophotometer and a spectrofluorophotometer can provide the pollution index of organic substances in water quickly. The absorption in the ultraviolet region is known to have good correlation with COD (chemical oxygen demand) and the absorbance near 250nm allows pollution by organic substances in water to be examined. Three dimensional fluorescence spectra have the capability to qualitatively and semi-quantitatively identify fluorescing substances in water with high sensitivity. In this report, we demonstrate the detection of source water contamination by organic substances in environmental water and tap water using a UV-Visible spectrophotometer and a spectrofluorophotometer.

An absorption spectrum of environmental water measured with a UV-Visible spectrophotometer has no absorption maximum or absorption minimum in the ultraviolet region and shows monotonous increase of absorption as the wavelength shortens. The absorbance spectra of river water sampled on May 9th and 11th showed monotonous increase of absorption in the ultraviolet region as mentioned above and their spectral shapes were similar to those of humic acid known as a corrosive substance dissolved in a NaOH aqueous solution or pure water in the longer wavelength region than 230 nm. Three dimensional fluorescence spectra of the environmental water measured with a spectrofluorophotometer showed signals around 430 nm of the emission wavelength (Em) while moving the excitation wavelength (Ex) in the range from 260 nm to 320 nm. This is thought to originate from corrosive substances included in river water. Three-dimensional fluorescence spectra of humic acid, whose peak position was different from that of the environmental water, showed a similar shape with that of the environmental water. The difference between the peak positions is considered to be due to the fluorescence characteristics depending on the structure of corrosive substances because each river water has a variety of forms for them. Additional signals derived from protein appeared near Em 340 nm in the three dimensional fluorescence spectra.

The daily measurement of these samples indicated the strong correlation between the absorbance at 254 nm and the three dimensional fluorescence spectra of corrosive substances. This work demonstrated that the use of a UV-Visible Spectrophotometer and a spectrofluorophotometer was capable of quickly evaluating the pollution status of organic substances in water.
**Abstract Text**

Detection of latent fingerprints using two classes of benzophenoxazine dyes (Nile red derivatives and Nile blue derivatives) was investigated. The hydrophobicity as characterized by the molecule’s logD value and chemical structure of a dye play a significant role in the ability to detect fingerprints. Nile red shows excellent ability to detect fingerprints on porous surfaces because of its high hydrophobicity. Using higher hydrophobicity dialkylated Nile red derivatives including WA2 and WA3 improves both quality and sensitivity of fingerprints detection. These dyes are considered as dual fingerprint reagents because they provide not only luminescent impressions but also visible fingerprints can be seen by a naked eye. However, low hydrophobicity dialkylated and monoalkylated Nile red derivatives display less luminescent fingerprints compared to that of Nile red when using the forensic light source at an excitation wavelength of 515 nm. Nile blue shows acceptable detection of fingerprints on porous surfaces. High hydrophobicity dialkylated Nile blue derivatives including VM38 and VM46 provide fingerprints that more luminescent and ridge detailed relative to that of Nile blue. Furthermore, visible fingerprint impressions are observed when using these dyes due to their dual ability to detect fingerprints. Nonetheless, monoalkylated Nile blue derivatives whether they are higher or lower hydrophobicity than that of Nile blue display less luminescent fingerprints compared to that Nile blue when using the forensic light source at CSS setting. A complete analysis of what dye properties are the most important in fingerprint detection are discussed along with suggested chemical structures for improved fingerprint detection.

**Keywords:** Fluorescence, Forensic Chemistry

**Application Code:** Homeland Security/Forensics

**Methodology Code:** Fluorescence/Luminescence
More than 200,000 emergency department visits each year in the United States are caused by food allergies, which affect nearly 5% of adults and 8% of children. The only current treatment for food allergies is a strict avoidance diet. To help affected consumers follow such diets, the Food Allergen Labeling and Consumer Protection Act of 2004 (FALCPA) requires labeling for the presence of specific allergenic foods. Enforcement of this regulation commonly relies on the detection of allergens using commercial antibody-based ELISA test kits. However, ELISAs only target a single analyte per kit, and cannot distinguish a positive result arising from the target analyte from cross-reactivity with homologous proteins present in the food sample. A solution to this problem is multiplex analysis, whereby the simultaneous use of multiple antibodies in a single assay enables the generation of an antigenic fingerprint or profile. Using a newly developed multiplex assay with up to 30 different antibodies against 14 different food allergens and gluten, we have analyzed the antigenic profile of a range of botanical dietary supplements and spices under both denatured and non-denatured extraction conditions. We have identified several cross-reactive species that could result in false positives in single analyte ELISA. To this end, we show how the use of an antigenic fingerprint produced with multiple different antibodies against an individual allergen makes it possible to distinguish cross-reactivity from the response of an actual food allergen.
Microchip Western blotting reduces sample volume, required time and challenges faced in analyzing complex protein systems by using a microfluidic chip for gel electrophoresis separation with direct capture of proteins onto a membrane. A microchip with an 8.6 cm long separation channel has been used for detection of 11 proteins from 9 injections of a single Jurkat cell lysate sample containing 400 ng total protein; each separation is completed in about 8 minutes. In comparison, conventional Western blotting techniques require 1-4 hours for separation and transfer while typically using 10-20 µg total protein. Traditional immunoassays used in Western blotting require 4-24 hours to complete and utilize large volumes of antibodies and reagents. Fast, microscale immunoassays can be achieved through pressure-driven flow deposition of antibody solutions onto the µm-wide protein trace as an X-Y stage moves the membrane with deposited proteins through. The use of vacuum during washing steps also decreases time requirements. The immunoassay methods for microfluidic Western blotting reduce antibody consumption, reagent consumption, and assay time for progress towards a more automated system for the microfluidic Western blot. In a model protein study with actin the microscale immunoassay achieves comparable signal-to-noise results in less than 2 hours with a 40-fold decrease in primary antibody consumption. Deposition of the secondary antibody by the optimized methods is under further investigation and the microscale immunoassay system is being applied to detection of proteins in cell lysates.

Keywords: Electrophoresis, Immunoassay, Lab-on-a-Chip/Microfluidics, Protein
Application Code: Bioanalytical
Methodology Code: Microfluidics/Lab-on-a-Chip
This paper describes a simulation study of a double-stacking lipid bilayer formed by a microchannel. Artificial lipid bilayers with membrane proteins are widely used as artificial models of cell membranes. Recent years, some groups have been reported miniaturized artificial lipid bilayer systems by using microfluidic technologies. However, the stability of the lipid bilayers is lower than conventional lipid bilayers formed by a droplet contact method. In this study, we proposed a microchannel which is built reservoirs for refueling lipids, and simulated lipid bilayer formation by using microchannels.

The microchannel consisted of three main flow channels, two sideways through the main flow channels and two reservoirs. First, the microchannel was filled with an organic solvent containing lipid molecules. Then, an aqueous solution flowed into all channels. As a result, lipid bilayers were formed on the sideways. The microfluidic simulations were done using the FEM based software COMSOL 5.2a. The interfacial tension between the aqueous solution and the organic solvent with lipids, the contact angle between the aqueous solution and the wall surface and the flow speed were set at 15 mN/m, 60° and 1 m/min, respectively.

As simulation results, we confirmed that oil layers remained in the sideways. This result indicated that lipid bilayer can be formed by using the microchannel with the oil reservoir. In the future plan, we will fabricate microchannels, and confirm the influence of the reservoir for the lifetime of lipid bilayers.

Keywords: Lab-on-a-Chip/Microfluidics, Lipids
Application Code: Bioanalytical
Methodology Code: Microfluidics/Lab-on-a-Chip
## A Precise Bead-Based Glutamate Quantification System Using Chip-Based Microfluidic Cytometry

L-Glutamate (Glu) is a vital amino acid which functions as an essential intermediate in amino group catabolism in most organisms. In particular, L-Glutamate is a dominant neurotransmitter in the brain and has relation to various fundamental neural processes. For these reasons, quantification of Glu concentration is meaningful in biological researches and clinical examinations. Chip-based flow cytometry is an important technology for its various capabilities like counting and characterizing particulates. Specially, DC impedance-based flow cytometry using our group's proprietary polyelectrolytic gel electrodes (PGEs) has advantages in small footprint and simplicity compared with other systems using optical and AC impedance. Here, we engineered intensity-based Glu-sensing fluorescent reporter so that the protein can be immobilized on streptavidin-modified magnetic microbeads and the microbeads serve as Glu sensor. The quantification of glutamate can be achieved by using DC impedance detection with polyelectrolyte gel electrodes (PGEs) and homemade optical detection system made up of photomultiplier (PMT) and an argon laser. The glutamate sensing microbeads were measured by both impedance and fluorescence detection simultaneously. When the microbead passes through the microchannel between the PGEs, DC impedance responses very sensitively for their size. The intensity of fluorescence has a correlation with the size of the microbead. According to the correlation, the intensity of fluorescence was calibrated by DC impedance signal. Therefore, quantification of glutamate was carried more precisely and quickly with chip-based microfluidic cytometry.

### Keywords
- Amino Acids, Bioanalytical, Lab-on-a-Chip/Microfluidics

### Application Code
- Bioanalytical

### Methodology Code
- Microfluidics/Lab-on-a-Chip
Microfluidic devices have the potential to make a significant contribution to stopping drug resistant bacterial infections by providing rapid diagnosis of the drug resistance profile of bacteria. The focus of our group's research is the integration of technologies suitable for this purpose into a miniaturized system. Specifically, we have been developing hybridization-based fluorescence detection of DNA sequences associated with drug resistance genes.

To create a microfluidic device capable of capturing, labeling and detecting sequences associated with drug resistance in bacteria, we have been conducting hybridization reactions in Eppendorf tubes as a preparatory step to working in a microfluidic environment. Using a Nanodrop instrument to measure fluorescence, we have shown that DNA attached to streptavidin beads captured complementary sequences at 100 nM from solution with ~80% efficiency. We have carried out these capture efficiency studies on 7 different targets. The effects of capture-oligonucleotide lengths on hybridization were studied to allow clinical samples to be analyzed. We have demonstrated ~95% recovery of captured DNA from the beads after denaturation. We have also investigated the use of molecular beacons to specifically label sequences. Finally, we have placed magnetic beads into microfluidic devices to create a format more amenable to scale up. Our preliminary results show strong evidence that microfluidic platforms are a promising solution to the problem of rapidly diagnosing the drug resistance profiles of infectious bacteria.

Keywords: Bioanalytical, Fluorescence
Application Code: Biomedical
Methodology Code: Microfluidics/Lab-on-a-Chip
Lab-on-paper-based devices or paper-based microfluidic devices ([micro]PADs) have emerged as inexpensive, disposable and portable analytical platforms for achieving rapid quantitative measurements of various analytes. Fabrication of [micro]PADs relies on patterning hydrophilic-hydrophobic regions on a sheet of paper. Wax printing method addresses well the simple, rapid and low-cost fabrication. A [micro]PAD design featuring a 96-well plate format was developed for the study of CYP3A4 activity using resorufin benzyl ether as an enzyme substrate. Since CYP3A4 is the most abundantly expressed CYP450 isozymes and it has also been involved in the drug metabolism as well as in clinically significant drug-drug interaction [1, 2]. Thus, incorporation of CYP3A4 enzyme screening at an early stage of drug discovery is preferable in order to avoid drug interactions. The optimal condition for enzymatic assay was in 50 mM potassium phosphate buffer (pH 7.4) containing 0.1 mM dithiothreitol, 1.3 mM NADPH and 0.02% poloxamer188, incubated at 37°C for 30 min. Measurement of CYP3A4 activity using the fluorescent detection at excitation/emission wavelength of 570/600 nm, on [micro]PADs format provided comparable results with those obtained from plastic 96-well plates. However, [micro]PADs offered higher sensitivity than well plates due to the greater facilitating the enzymatic reaction. Moreover, [micro]PADs (cost~$0.1/[micro]PAD) is more cost effective than plastic well plates (cost~$2.6/plate). The optimal condition was then transfer to determine the enzyme activity on [micro]PADs using handheld fluorescence microscope. The fluorescence intensity can be accurately quantified by taking a photo of the test zone measuring the intensity by image analysis freeware (ImageJ). The limit of quantitation was 0.71 µM with good precision (RSDs < 5.2%). Moreover, CYP3A4 was immobilized in sol-gel formulation containing 8.3 M Ludox® TM-50 colloidal silica and 0.125 M sodium silicate solution with the ratio of 1:2, then coated on µPADs. The immobilized enzyme was stable for at least 1 week at 4 [degree]C, which offered an increased storage stability comparing to the free solution. The developed [micro]PADs provide simplicity, low cost and portability and approaches green analytical chemistry. The method would be useful for assessment of potential new drug candidates.

Keywords: Bioanalytical, Biosensors, Fluorescence, Lab-on-a-Chip/Microfluidics
Application Code: Bioanalytical
Methodology Code: Microfluidics/Lab-on-a-Chip
Resonance acoustic waves in microfluidic devices have been well utilized to concentrate and separate cells and microspheres. These resonance waves generate size dependent acoustic forces on particles. It is easier to manipulate larger particles and cells very effectively in an acoustic field. However when particle gets smaller (< 1 µm in diameter), acoustic forces become weaker and manipulation of cells and particles becomes challenging. On the other hand if two miscible fluid streams with relatively different densities flow through a microchannel as a laminar flow, acoustic forces can deform the liquid-liquid interface. We have used this phenomenon to switch the path of two parallel fluid streams in a microchannel. Further, by combining acoustic focusing of particles and acoustic flow switching we have developed a new acoustofluidic method to separate nano particles (<500 nm) from micro particles very efficiently. The proof of the concept is demonstrated in an acoustic flow channel fabricated in a silicon wafer. These channels were created using chemical etching techniques. For samples, we have used mixtures of nano and microparticles as well as biological samples containing bacteria and cells. Data were collected as fluorescence images obtained via an epi-fluorescence microscope. We envision that our approach can be further developed into a density gradient acoustic fractionation technique for separation of similar cells with different densities. <B>Acknowledgment: </B>Funding for this work was provided through Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant number P20GM103451.

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

**Application Code:** Bioanalytical

**Methodology Code:** Microfluidics/Lab-on-a-Chip
Polydimethylsiloxane (PDMS) has been used to create microfluidic devices for cell culture purposes. However, one of the limitations of PDMS is that it absorbs hydrophobic molecules such as pharmaceutical drugs, proteins, and small molecules that have a large partition coefficient. For studies that involve the use of a fixed amount of solution to measure cell response, the absorbance of hydrophobic molecules can result in an incorrect amount of exposure to the cell, adversely affecting the outcome. We present a simple fabrication of microfluidic devices made out of polyethylene terephthalate (PET) to reduce the absorbance hydrophobic molecules. Our typical microfluidic devices consist of a two-chamber PDMS device bonded to a glass slide by surface treatment method using a plasma cleaner. A polymer membrane is sandwiched between the two PDMS layers. The device contains one channel in the top layer and another in the bottom layer for cell culture and fluid movement. We have modified the device fabrication such that the area of PDMS that is exposed to the fluids is reduced by over 90%. Briefly, we precisely laser cut PET and solvent bonded to the device layers to minimize the use of PDMS in the device. As most of the contact with PDMS is removed, we were able to minimize the impact of the absorption. We verified the reduction in absorbance of fluorescently labeled molecules. The simple fabrication technique does not require expensive tooling such as molds that are needed for fabricating plastic microfluidic devices. This research will limit the effect of small molecule absorption in many microfluidic applications by using PET as an alternative of PDMS.

This work was supported in part by NIH grant DK095984.
Diabetes is a metabolic disease characterized by a hyperglycemic state, which, if left unmanaged, can generate reactive oxygen species (ROS). ROS will attack endothelial cells, which line the walls of blood vessels, and cause cardiovascular damage. We have created an \textit{in vitro} system that seeks to model the metabolic state due to hyperglycemia \textit{in vivo}. Our system is a microfluidic cell culture platform which uses microchannel dimensions that accurately mimic a blood vessel. This system provides faster lysing time and reduced reagent consumption which helps limit the loss of information prior to analysis. The device is able to provide an automated cell lysis system which can achieve complete cell lysis in 15 seconds with conventional lysing solvent alone, and within 4 seconds when combined with in-channel electroporation. Reducing the temperature to 4.5°C during lysis and sample collection with a cooling component reduces enzyme activity to that of conventional lysing without using centrifuge methods. The analysis of cell lysate resulted in identification of several species relevant to diabetic complications. This work will investigate the damage hyperglycemia does to metabolic pathways which will aid in the elucidation of potential therapeutic targets.

Funding for this project was provided by grants from the American Heart Association, National Institutes of Health, and Saint Louis University.

\textbf{Keywords:} Bioanalytical, Metabolomics, Metabonomics

\textbf{Application Code:} Bioanalytical

\textbf{Methodology Code:} Microfluidics/Lab-on-a-Chip
Nucleosome preparation is important for many epigenetic studies including chromatin immunoprecipitation (ChIP). ChIP is the gold standard for probing epigenetic protein-DNA interactions that plays critical roles in cell fate and function, aging and carcinogenesis. While developed and applied for many years, traditional ChIP protocols require a large cellular input (10^6-10^7 cells) to prepare target nucleosomal DNA, which limits their utility to study biopsies, rare cells such as cancer and stem cells, and to assess tumor heterogeneity. The nucleosome preparation in ChIP is also laborious and time-consuming, and user-dependent. To address these disadvantages, we have developed an automated, droplet-based microfluidic device that prepares nucleosomal DNA directly from cells within half an hour, more rapidly than traditional protocols. With this device, starting number of cells and reaction time are flexible. Percentages of mono-, di- and tri-nucleosomes are adjustable to satisfy the requirement of different downstream ChIP processing with the capability to accurately control on-chip incubation time. This enabling device is also capable to be combined with other microfluidic modules such as magnetic beads-enabled chromatin capture, thus suitable for many epigenetic study assays, including nucleosome positioning assay, ChIP, and DNase I hypersensitive sites assay. This device will provide unprecedented opportunity to prepare samples for studies of multiple epigenetic profiles down to the level of single cells allowing both the assessment of cell heterogeneity within complex clinical samples and the application of cost-effective epigenetic testing to very small samples in individualized medicine settings directly at the point of care.

This work is supported by Mayo-Illinois Alliance for technology based healthcare, National Cancer Institute (R21CA 191186), and NIH-sponsored Midwest Cancer Nanotechnology Training Center.
Microfluidic Methods

Continuous In-Droplet Sample Washing: An Emerging Tool for Chemistry in Picoliter Droplets

Droplet microfluidic devices produce libraries of individually addressable reactors by segmenting samples into microdroplets surrounded by oil. While this approach increases mixing speed, reduces sample loss, and enables precise handling of especially small or rare samples, fully realizing sophisticated in-droplet chemistries requires a toolkit for controlling the chemical environment of the droplet. We have recently described the “K-channel,” a module which interfaces a continuous flow with the segmented droplet flow to modify droplet contents. Unlike traditional, geometry-constrained approaches, this single device injects into droplets, extracts from droplets, and splits droplets, among selected operations, depending on applied conditions like pressure and electric field. Recently, we have extended serial combinations of this module toward sample washing. By splitting antibody-functionalized magnetic bead-laden droplets at the first K-channel and reinjecting buffer into split droplets at the second K-channel, we alter droplet composition. Importantly, the presence of an external magnetic field concentrates bead-bound samples into the portion of each droplet retained during droplet splitting. By capturing beads while exchanging droplet contents, we can successfully achieve the sample washing steps required by many chemical and biochemical assays at 200 droplets per second, a frequency more than an order of magnitude faster than previously reported in droplets. Current work continues to adapt this approach as a platform for biochemical techniques such as immunoprecipitation, microextraction, and more.

This work is supported by NIH 1R21CA191186-01, a Robert C. and Carolyn J. Springborn Graduate Fellowship, and an NSF Graduate Research Fellowship.

Keywords: High Throughput Chemical Analysis, Lab-on-a-Chip/Microfluidics, Sample Handling/Automation, Solid
Application Code: High-Throughput Chemical Analysis
Methodology Code: Microfluidics/Lab-on-a-Chip
Droplet microfluidics is an attractive technology for chemical and biological research due to their small sample volume and low sample loss. Unlike conventional methods, such as an Enzyme-linked immunosorbent assay (ELISA), droplet microfluidics can provide higher throughput screening for faster analysis. Recently, we have identified an approach to single enzyme molecule detection via fluorescence within droplets. A key feature of the device is that each droplet either has one or no analyte by using very dilute analyte concentration. Thus, the concentration of substrate can be measured digitally compared to total analog signal. Therefore, the number of droplets that fluoresce is equal to the number of target molecules in sample. We have developed a continual flow device that can detect single β-galactosidase enzymes within droplets. With the utilization of the “K-channel,” reagents can be injected into droplets, extracted from droplets, and droplets can be split. By altering the applied pressure and electric field, we can alter the performance of the K-channel to perform one operation to another. The ability to manipulate droplets allows us to perform washing steps mechanically without human error. Furthermore, with the K-channel, we can fully remove droplets. This allows us to separate droplets with enzyme and droplets without enzymes. Our current work has potential to be used as a method of determining protein concentration at a lower limit of detection in comparison to other conventional techniques.

**Keywords:** Bioanalytical, Enzyme Assays, Lab-on-a-Chip/Microfluidics, Single Molecule

**Application Code:** Bioanalytical

**Methodology Code:** Microfluidics/Lab-on-a-Chip
Colorimetric enzyme linked immunosorbent assay (ELISA) is one of the most conventional laboratory assays for medical diagnostics, quality control and research applications. However, unspecific absorption of protein may lead to reduction of functional sites, resulting in high background and low sensitivity in ELISA. Herein, we report a simple method of functionalization of poly(methyl methacrylate) (PMMA) with polylysine, which can be used as a substrate for ELISA with high amine density, for high-sensitivity multiplexed detection of biomarkers. Covalent binding of antibody/antigen to amine dense microplate resulted in a significant improvement of sensitivity as compared to traditional microplates. Results of the assay can be viewed by naked eyes or scanned through a simple desktop scanner for quantitative analysis within 90 min. As a proof-of-concept for multiplex detection, we carried out the sandwich-type immunoassay for the simultaneous detection of immunoglobulin G (IgG), hepatitis B surface antigen (HBsAg), and hepatitis B core antigen (HBcAg). Without the use of any specialized equipment like microplate reader, limits of detection (LOD) of 200 pg/mL, 180 pg/mL, and 300 pg/ml were obtained for IgG, HBsAg, and HBcAg, respectively. The surface modified microplate was found to be at least 10 fold more sensitive than traditional microplates. The device can be used for low-cost and reproducible detection of infectious diseases, cancers, and other biomolecules.

Financial support from NIH, UT STARS Award, MRAP, BBRC, IDR2 and URI award from UTEP is gratefully acknowledged.

Keywords: Bioanalytical, Immunoassay, Lab-on-a-Chip/Microfluidics, Polymers & Plastics
Application Code: Bioanalytical
Methodology Code: Microfluidics/Lab-on-a-Chip
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.
Microfluidic Methods

Nanoelectrospray Ionization-Mass Spectrometry Analysis of Droplets Containing Ion Suppressing Matrices

The development of microfluidics has allowed for the manipulation of small volumes of liquid for a variety of applications. By dividing flow with an immiscible carrier phase, discrete samples can be created with volumes in the nanoliter range and below. Chemical analysis in droplet microfluidics is often performed by mass spectrometry (MS), which can analyze the content of droplet samples directly, without any need for labeling. Electrospray ionization (ESI) is a choice ionization technique for MS in droplet microfluidics, as it can create gaseous analyte ions directly from liquid sample. One problem commonly observed in ESI-MS is the presence of matrices that drastically reduce the formation of gas phase analytes. In this work, we examine the use of nanoESI-MS (nESI-MS) for the direct analysis of droplet samples containing ion suppressing matrices. By lowering flow rates and spray tip sizes well below that of conventional ESI, nESI exhibits higher tolerance towards dirty matrices. We have demonstrated the feasibility of using nESI-MS for droplet analysis by examining the neurotransmitter content of long (>100 droplets) trains composed of ion suppressing artificial cerebral spinal fluid at flow rates as low as 20 nL/min. Future work will examine the attainable limits of detection for neurotransmitters of interest, as well as working towards minimizing droplet size and analysis time. Droplet nESI will then be applied for the purpose of chemical sensing in biological systems, as well as high-throughput drug screening of relevant biological targets.

This work is supported by NIH grant #R01GM102236.

Keywords: Electrospray, Lab-on-a-Chip/Microfluidics, Mass Spectrometry, Tandem Mass Spec
Application Code: Bioanalytical
Methodology Code: Microfluidics/Lab-on-a-Chip
Lab on a tablet concept is proposed a design policy of optical system that is combined with a personal information “tablet”. [1] Polydimethylsiloxane (PDMS) is suitable for the concept owing to its flexibility, glass-adhesiveness, small autofluorescence and UV transparency. An attachment of monolithic PDMS module, that has partially dispersed carbon particle [2], pasted on the glass surface of an LCD of the tablet can trap a stray light propagating between the glass surface and the PDMS.

The optical component between chamber/mounter of PCR tube for an absorption measurement was fabricated by the 10 wt.% CB:PDMS (figure), and it can suppress light scatter between the component. The waveguiding from the LCD light source to the face camera was performed using a polymeric optical fiber. Then an illuminated fiber’s end faced camera was captured. Then captured and circled light spot was cropped by an optimized threshold, which is calculated by discriminant analysis method with an Otsu algorism. [3] Finally the absorbance was obtained from the averaged intensity of the cropped area, and it shows no linearity for the concentration. According to experimental result, the concentration down to the 0.1 [micro]M can be expected. [4]

As the result, the slight misalignment in the PCR tube mounting operation may affect the measurement stability. Therefore, the captured images were processed and analyzed. Firstly, a gradient-color conversion was applied, and the image was circumferentially averaged. Since the obtained circle image’s edge intensity showed difference when the PCR is misaligned, the cross-section profiles were compared and plotted. This examination protocol can be expected to reduce affection of the insertion misalignment.


Keywords: Data Analysis, Lab-on-a-Chip/Microfluidics, Software, UV-VIS Absorbance/Luminescence
Application Code: Environmental
Methodology Code: Microfluidics/Lab-on-a-Chip
Microfluidic Methods

A Customizable 3D-Printed Equilibrium-Dialysis Device for Enhanced Binding Studies

Measuring the binding affinity of drugs, ions, and hormones to serum proteins is of major importance in pharmacology because it provides information about the bioavailability of these analytes to cells and tissues. Equilibrium dialysis is a technique that has been used for many years to measure the fraction of a ligand bound to a protein. Commercially available equilibrium-dialysis devices have several limitations, including cost, incompatible membrane material, and limited pore size options. Here, we used 3D-printing to create our own customizable equilibrium-dialysis device, capable of measuring the binding of different ions, molecules, and hormones to proteins and cells. This innovative device enables various binding experiments by choosing the optimum membrane material and pore size. The device was used to measure the binding affinity of different important molecules in diabetes and albumin, an abundant carrier-protein in the bloodstream. High blood-glucose concentrations in diabetes cause glycation of serum albumin, altering its ability to bind important ligands and causing a change in the bioavailability of these ligands to cells and tissues. Specifically, the new device was used to measure the change in affinity between different ligands with normal and glycated albumin. The new device is available for public use via online file-sharing websites.

Keywords: Biotechnology, Lab-on-a-Chip/Microfluidics, Membrane, Pharmaceutical
Application Code: Bioanalytical
Methodology Code: Microfluidics/Lab-on-a-Chip
Counting the Dots: A Novel Approach to Quantitative Data Acquisition in Wicking Microfluidic Analytical Devices

Cost and complexity of device fabrication and assay detection are key factors in designing wicking microfluidic devices for applications of all kinds. Although a significant amount of work has been done to reduce the cost of device fabrication, most of the assay detection mechanisms still depend on complex procedures and require use of external instrumentation. This results in devices that necessitate specific conditions, facilities, and trained personnel. Such dependencies not only increase the cost of analysis but also limit the usability of these devices in field applications. Therefore, it is important to develop an accurate and easy-to-use qualitative and quantitative assay detection process that is largely, if not entirely, stand-alone capable.

Here we present a novel, stand-alone approach to quantitative determination of analytes based on dot-counting. This detection approach allows for the use of non-specific assay chemistries to detect multiple analytes based on variations in solubility of metal ion/chelator complexes. In this way, interferences are kept to a minimum without the use of additional chemistries or instrumental/analytical approaches. The unique fabrication method employed allows the concentration range to be tuned by controlling the wicking channel geometry (width). The accuracy and limit of detection can also be easily adjusted by increasing or decreasing the resolution of the inkjet dispensed assay dots without the need for further fine tuning of the reagent composition. The capabilities of this unique combination of detection mechanism and device fabrication have been demonstrated using a polymer inclusion membrane (PIM) based assay design to detect aqueous heavy metal ions.

References

Keywords: Environmental Analysis, Lab-on-a-Chip/Microfluidics, Polymers & Plastics
Application Code: Environmental
Methodology Code: Microfluidics/Lab-on-a-Chip
White adipose tissue (WAT) is now considered as a complicated endocrine organ able to produce and secret several bioactive factors related to multiple metabolic abnormalities such as obesity, diabetes, arterial hypertension, Alzheimer’s disease, and many other diseases. Diabetes, especially Type II diabetes, is highly correlated with obesity, largely a result of insulin resistance and beta-cell dysfunction. Other groups have shown that the two-dimensional co-culture of differentiated 3T3-L1 adipocytes with primary rat islets caused beta-cell dysfunction, including the decrease of insulin secretion and insulin content, as well as reduced mRNA expression of GLUT2, GCK and Kir6.2. However, only limited information is available concerning adipokine secretion and nutrient uptake dynamics in such co-culture. We have previously developed 3D printer templated microfluidic devices for culture and sampling of both primary murine islets and adipose tissue explants, as well as homogeneous assays for insulin and cAMP based on thermofluorimetric analysis. Here, we present a new microfluidic device for the co-culture of primary WAT and primary islets (Figure 1A-B) to investigate dynamic interactions between the tissues related to hormone secretion and nutrient uptake. The explant culture well and the islet culture well were sculpted into the polydimethylsiloxane (PDMS) device using 3D-printed templates. Preliminary results showed that peaks of insulin secretion from islets correlated with valleys in glycerol secretion. The combination of this newly designed chip with our novel homogeneous assays allows us to further understand the interaction of islets and adipose tissues.
Currently affecting more than 25 million Americans (10% of all US adults), chronic kidney disease is a growing public health concern, and is the ninth leading cause of death in the world. Since adults with diabetes or high blood pressure have a higher risk of developing chronic kidney disease than the healthy individuals, and given that the number of civilians diagnosed with diabetes or hypertension is continuously increasing, it is expected that even a larger population will be affected by chronic kidney disease. Currently, patients need to frequently visit the primary care center to submit blood or urine samples; the samples are then sent to the central laboratories for analysis of electrolyte levels and bio-markers, and the results are reported back to the physician. This process is time-consuming and inconvenient for patients and diagnosis by the physician is deferred until later access to the results of the tests. A companion diagnostic technology that provides rapid (within minutes), reliable, and low-cost measurement of electrolyte levels or bio-markers in blood or urine samples at the point-of-care or patient’s home is needed. Paper is an appropriate substrate for development of such diagnostic devices because it is low-cost, is widely accessible, and has the capabilities to store materials and transport biological fluids in defined microfluidic channels. In this work, we are developing a disposable single-use paper-based device for electrochemical monitoring of bio-markers of chronic kidney disease.

Keywords: Analysis, Bioanalytical, Biosensors, Electrochemistry
Application Code: Biomedical
Methodology Code: Electrochemistry
Exploring Energy Transfer in Pt Decorated Au Nanoprisms via Electron Energy-Loss Spectroscopy

Driven by the desire to understand energy transfer between plasmonic and catalytic metals for applications such as plasmon-mediated catalysis, we present a study comparing pure Au nanoprisms to Pt-decorated Au nanoprisms. Surface plasmon spectra and mode maps acquired through electron energy-loss spectroscopy (EELS) reveal detailed near-field information on the coupling and energy transfer in these systems, elucidating the mechanisms by which plasmon-driven chemistry occurs in mixed-metal nanostructures. Through a combination of experiment and theory we demonstrate that, although the location of the Pt decoration greatly influences the plasmons of the nanoprism, simple spatial proximity is not enough to induce significant energy transfer. What matters more is the spectral overlap between the intrinsic plasmon resonances of the Au nanoprism and Pt decoration, which can be tuned by changing the composition or morphology of either component.

Keywords: Energy, Material Science, Spectroscopy
### Abstract Text

A calibration model in attempt to enhance LAMIS for its viable capabilities in sensitivity (down to ppm) and discrimination in isotopic analysis is presented here. The radiative transitions from molecular species along with the generated single shot spectra of zirconium particles ranging for 45-200 μm in diameter was dispersed on the surface of a silica substrate were used as the predictors for the model. For the analyte(s) of interest, the generated plasma using a Q-switched 1064nm Nd:YAG excitation laser has been examined for their ZrO molecular bands. To ensure single particle spectra, the laser beam was focused to spot size of 100 μm on the sample and a CCD system was employed for imaging single particle and laser interaction. A minimum of two band heads in the following systems [β] B3[3]/sup]/sup]Δ2A[3]/sup]Δ [3]/sup]/sup]Δ X [3]/sup]/sup]Δ 3682 [sup]1[/sup]/sup]Δ[sup]1[/sup]/sup]Δ[3]/sup]Δ transitions have been identified. The data sets were analyzed using partial least squares regression (PLSR) in an effort to determine the isotopic concentration of [90]/sup]Zr and [94]/sup]Zr in a zirconium oxide mixture.

### Keywords
- Laser, Molecular Spectroscopy, Nuclear Analytical Applications, Trace Analysis

### Application Code
- Nuclear

### Methodology Code
- Molecular Spectroscopy
It is essential to understand the effects of electrode surface heterogeneity at the nanoscale to study electrochemical processes in a number of fields including biological electron transfer, electrocatalysis, battery, and energy storage. We developed a tip-enhanced Raman spectroscopy (TERS) method based on AFM platform to study electrochemical reactions at the single-molecule level occurring at nanoscale surface sites. Here, we present TERS cyclic voltammetry measurements based on an AFM platform to investigate local electrochemical behavior of single- to few- molecules. Statistical simulations and a fitting procedure for describing single-molecule voltammograms and single charge transfer events provide thermodynamic and kinetic parameters for a single molecule. Both TERS cyclic voltammetry and statistical simulations provide a strong methodology for bridging the electrochemical behaviors of a single molecule to existing knowledge of ensemble-averaged electrochemistry.

**Abstract Text**

**Keywords:** Electrochemistry, Molecular Spectroscopy, Raman Spectroscopy, Spectroelectrochemistry

**Application Code:** Nanotechnology

**Methodology Code:** Molecular Spectroscopy
A sensitive Raman optical probe was produced by incorporating porous surface-enhanced Raman scattering (SERS) substrate with optical fiber. The SERS substrate used in this work was fabricated by decorating silver nanoparticles (AgNPs) on porous polymelamine with wet chemical method. Meanwhile, the fiber-optical sensing tip was varied by itching 550-μm diameter quartz fibers in HF solution with different conditions. After placing SERS substrate to the sensing tip of optical fiber, a SERS fiber-optic sensing probe was successfully produced. SERS sensing probes were further characterized by detecting adenine in aqueous solution. Significant improvement of sensitivity was observed as at least 8 times of sensitivity was obtained compared to the literature reported fiber type of SERS probes. To optimize this sensing system, both influences of the condition in preparation of SERS substrate and the geometry of optical fibers were systematically examined. With the optimized preparation condition, SERS fiber-optic sensing probe with a detection limit of 5 nM was obtained for detection of adenine and its linear range was up to 50 μM. Application of SERS probe in determination of adenine in urine samples showed that adenine could be determined accurately with a recovery ranged from 93.9% and 110.0%.

Keywords: Surface Enhanced Raman Spectroscopy
Application Code: Bioanalytical
Methodology Code: Molecular Spectroscopy
The levels of methylated metabolites in biological systems play crucial roles in the cellular metabolic pathways and the responses to environmental stresses. To determine the levels of methylation of some important metabolites, a rapid and sensitive method based on surface-enhanced Raman spectroscopy (SERS) was developed. In this method, SERS active substrate was fabricated by decorating silver nanoparticles (AgNPs) on 3-dimensional template of cellulosic fibers. The polar nature of the template increases the ability for detection of analytes in aqueous solution, while the AgNPs largely improve the sensitivity in detection. To successfully decorate AgNPs on cellulosic fibers, cellulosic fibers were first treated with base to effectively adsorb AgNPs produced by a chemical reduction method. The condition to prepare the substrate was optimized using D-pinitol and [i]myo[/i]-inositol as probe molecules. With optimized preparation condition, the formed substrate offers an enhancement factor around 6 orders in magnitude with a relative standard error less than 10%. To demonstrate the applicability of prepared SERS substrate for detection of methylated sugar alcohols, the extracts the leaves of [i]Mesembryanthemum crystallinum[/i] in different growth stages were used for detection. With a simple clean up step, the level of D-pinitol could be accurately determined with a standard addition/dilution method.

Keywords: Surface Enhanced Raman Spectroscopy
Application Code: Bioanalytical
Methodology Code: Molecular Spectroscopy
The study of heterogeneous charge transfer is paramount in several fields, including biology, electrocatalysis and energy storage. State of the art performance is limited by the information available regarding the site specific behavior of electrodes at the nanoscale. In order to build structure-activity relationships for charge transfer reactions, we have developed an electrochemical version of Tip Enhanced Raman Scattering (EC-TERS) using an STM platform. TERS is a vibrational spectroscopy technique that can achieve nm resolution and is capable of single molecule sensitivity. Additionally, in EC-STM-TERS, the tip and the electrode are both under potential control. We have studied a model electron transfer reaction with Nile Blue on gold, both in the physisorbed state and covalently tethered to the surface. With this system, we demonstrated the acquisition of TERS during a charge transfer reaction at the single molecule level and monitored the oxidation state of the molecule. We studied phthalocyanines assembled on a flat gold electrode and spectroscopically addressed the central ion oxidation state in situ at the electrode surface. In particular, we monitored the charge transfer reaction occurring on Co and Fe phthalocyanines. These results allowed us to compare ensemble level measurements using cyclic voltammetry (CV) with nanoscale measurements using TERS to discuss single molecule kinetics as well as activity variations among sites on the electrode.

The authors acknowledge support from the AFOSR (MURI #FA9550-14-1-0003).

Keywords: Electrochemistry, Raman Spectroscopy, Spectroelectrochemistry, Surface Analysis
Application Code: Nanotechnology
Methodology Code: Vibrational Spectroscopy
Sub-terahertz (sub-THz) vibrational spectroscopy for biodetection is based on specific resonance features and vibrational modes or groups of modes at close frequencies that are in the absorption (transmission) spectra of large biological molecules and intact bacterial cells and spores. Improvements in sensitivity, especially in the discriminative capability of sub-THz vibrational spectroscopy for detection, characterization, and identification of bacterial organisms, require higher spectral resolution to resolve the spectral features. Herein we describe a new, continuous-wave and frequency-domain spectroscopy sensor with imaging capability. This spectroscopy system was operated at room temperature in the sub-THz spectral region between 315 and 480 GHz. We present experimental spectra obtained from biological macromolecules and species using this spectrometer, and we compare biological spectra with simulation results using molecular dynamics. Observed, multiple, intense and specific resonances in transmission and absorption spectra from nanogram samples with spectral line widths as small as 0.1 cm provide the conditions for determining reliable discriminative capability and for monitoring interactions between biomaterials and reagents in near real time.
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 tackle the challenges associated with NMR and MS and thus provides more complete coverage of metabolome. Here we demonstrate a high-throughput coupling of capillary LC with SERS (LC-SERS) using an online sheath flow SERS detector for analysis of metabolites in complex biological mixtures. We will also discuss the ability to detect and quantify analytes over a wide range of concentrations by utilizing both built-in UV-Vis detector in LC system and SERS in flow. A mixture of model metabolites (thiamine, folic acid, and riboflavin) was separated and characterized by UV-Vis and SERS detectors connected in series. Acetonitrile in the mobile phase provided an internal standard enabling quantitative detection across SERS experiments. Our results demonstrate that sheath-flow SERS is provides improved detection of molecules which suggests a complementary technique to identify and quantify small molecules separated by LC and can be run in parallel with MS based method.
Single molecule (SM) spectroscopy is a valuable endeavor towards the ultimate limit of detection and elucidating the heterogeneity of chemical reaction dynamics. Single molecule surface-enhanced Raman spectroscopy (SMSERS), in particular, is capable of both high spectral sensitivity and characterization of the molecule’s structure. Currently, wider application of SMSERS is hampered by challenges in nonresonant molecule detection and enforcement of reliable SM proof standards. First, we experimentally examined the chemical enhancement mechanism of SERS as a strategy to compensate for the resonance signal enhancement found in most current SMSERS experiments. Second, we investigated the molecular property differences that can lead to misinterpretation of the bianalyte proof for SMSERS. Utilizing a joint Poisson-binomial model and experimental results, we established the first set of viable experimental considerations and threshold standards for dependable proof of SM detection. With expansion of the SMSERS molecular library and further development of SM proof standards, SMSERS can grow as a highly sensitive analytical technique with powerful applications in surface science.

Keywords: Nanotechnology, Single Molecule, Surface Analysis, Surface Enhanced Raman Spectroscopy
Application Code: Nanotechnology
Methodology Code: Vibrational Spectroscopy
There is a strong need to move away from the conventional fossil fuels used today. Hydrogen is a promising alternative and its use will significantly reduce harmful emissions if produced by renewable energy sources. The performance of PEM fuel cells operated in for example vehicles is strongly dependent on the quality of the hydrogen gas, especially so with respect to (low level) impurities present. To ensure that the quality of the hydrogen gas meets the criteria specified in ISO 14687-2: 2012, the hydrogen gas has to be analyzed for the presence of these impurities. Many of these compounds have maximum allowable levels at nmol/mol level posing stringent requirements to the analytical instrumentation and sampling system.

An overview will be given of the development of the analytical capabilities at VSL with respect to the preparation and analysis of amongst others H2S, formaldehyde, formic acid and CO at nmol/mol levels in hydrogen. The focus will be on the use of GC-SCD for H2S detection and CRDS for measurement of CO, formaldehyde and formic acid.
Molecular Spectroscopy Advances: Raman and Infrared

Substrate Effects on Chemical Vapor Sensing with Single- and Few-Layer WS\(_2\)

Semiconductors have been used for chemical sensing due to their high sensitivity and low cost. Recently, single- and few-layer transition metal dichalcogenides (TMDCs) have been studied as chemical sensing platforms due to their high surface-to-volume ratio and semiconducting properties. One aspect of these materials that intrigues us is that the observed response time for a single-layer platform to gaseous analytes is remarkably slow (10s of minutes). To explore this issue, we use co-localized atomic force microscopy, Raman, and photoluminescence mapping of single- and few-layer WS\(_2\) flakes on sapphire, SiO\(_2\)/Si, Au, and free standing. This presentation will summarize our mapping results. This research was supported by the National Science Foundation.

Abstract Text

Keywords: Atomic Force Microscopy (AFM), Raman Spectroscopy, Sensors, Vibrational Spectroscopy

Application Code: Other

Methodology Code: Vibrational Spectroscopy
In recent years, the optical excitation of plasmon resonances in metal nanostructures has been shown to drive catalytic reactions. These photocatalytic effects have been demonstrated, but the underlying mechanisms behind why the reaction is taking place are unclear. Are the plasmons really driving the reaction or is something else going on at the surface of these nanostructures? Our research aims to understand how plasmons impact surface reactivity. Previous work has shown, through the use of Raman spectroscopy, that metal nanostructure SERS substrates induce a Stark Shift through a process known as optical rectification (using light to convert an AC field to a DC bias). This optical rectification is associated with the excitation of localized surface plasmon resonances (LSPRs) on a SERS substrate. By applying a bias voltage to counteract the shift, the intensity of the electric field produced by these LSPRs can be quantified. The origin of the bias is confirmed through complementary second harmonic generation (SHG), which arises from second order susceptibility similar to optical rectification. Using a reporter molecule to determine electric field strength, a reaction molecule to monitor chemical reactivity, and mapping along the surface with Raman, the LSPR strength on the surface potential can be correlated with possible catalytic activity. These experiments suggest new insights into the mechanism behind plasmon-mediated catalysis.

Keywords: Raman Spectroscopy, Surface Enhanced Raman Spectroscopy, Vibrational Spectroscopy
Application Code: Other
Methodology Code: Vibrational Spectroscopy
Much recent interest has been focused on the controllable synthesis of one-dimensional nanostructures such as nanorods and nanowires due to their unique and enhanced properties and great variety of potential applications. The ability to control the size of such nanostructures is very important since it dictates the materials characteristic properties. In this presentation, the one-pot synthesis with in-situ preconcentration of calcium sulfate nanorods (CSNR) via use of thermoresponsive nonionic surfactants based upon their phase behavior (cloud point extraction, CPE) will be described. CSNRs of different sizes can be obtained depending upon whether the synthesis/CPE is conducted in the presence or absence of added spherical silver nanoparticles (AgNPs). When Ca$^{2+}$ and SO$_4^{2-}$ are added (at concentrations below their solubility product) to a solution of the nonionic surfactant and CPE conducted, wire-like micrometer-sized CaSO$_4$ nanostructures (without any branches or aggregates) are concentrated in the surfactant-rich phase. If the same procedure was conducted in the presence of 30 nm AgNPs, then the length of the CaSO$_4$ rods shorten and the AgNPs were co-extracted into surfactant rich phase along with the CaSO$_4$ nanorods. The factors that impact this synthesis and the characterization of the formed CaSO$_4$ nanostructures will be presented and discussed.

**Keywords:** Metals, Nanotechnology, Surfactants, Temperature

**Application Code:** Nanotechnology

**Methodology Code:** Separation Sciences
Having found applications in a large range of consumer products such as e.g., white wall paint, cleansers or sunscreen formulations, TiO2-nanoparticles have nowadays become an essential part of our everyday life. However, in order to ensure a consistently good product quality as well as reliable safety and risk assessments, a comprehensive characterization of these engineered nanomaterials is indispensable.

In this presentation, we describe the application of Asymmetrical Flow Field-Flow Fractionation (AF4) coupled with MALS and DLS for the comprehensive characterization of commercially available TiO2-nanoparticles (AeroDisp® w740x from Evonik Industries, Germany) both in their native suspension as well as in a custom-made sunscreen formulation. Latter sample thereby represents a spiked real-world matrix, which has been pretreated with supercritical CO2 prior to AF4-analysis.

The determination of the radii of gyration as well as hydrodynamic radii facilitated the direct characterization of the applied TiO2-nanoparticles with particular respect toward agglomeration. Hence, the obtained results not only clearly indicate the excellent applicability of AF4 as a reliable technique suitable for the routine analysis of TiO2-nanoparticles during and after the manufacturing process, but also for the sensitive determination of TiO2-nanoparticles in sunscreen formulations. The capability of determining TiO2-nanoparticles with geometric diameter below 100 nm in real-world samples thus renders Field Flow Fractionation a promising tool for the monitoring of existing and upcoming EU regulations, where nanoparticle containing consumer products have to be specifically labeled.

Keywords: Extraction, Light Scattering, Nanotechnology, Separation Sciences
Application Code: Nanotechnology
Methodology Code: Separation Sciences
Study of adsorption of L-amino acids on metal surfaces (1) is of interest owing to their applicability to molecular electronics and biocatalysis (2). L-Serine is a polar amino acid containing an hydroxy group, which is capable of forming intermolecular hydrogen bonds to varying extent. Scanning Tunneling Microscopic (STM) images of L-serine adsorbed on Cu(111) at 55 K and 4.3 x 10e-12 Torr showed chain, two dimensional and various self assembled molecular clusters. Varying degrees of hydrogen bonding between the alcohol groups leading to two, three and four molecular crowns centered on Cu are believed to take place on the copper surface. Using the STM images, models for the formation of ordered phases with specific one-dimensional and two-dimensional molecular alignment are proposed.

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(1) Guisinger et. al. Nanotechnology 26 (2015) 235604  
Graphene has distinctive physical and chemical properties due to its unique spatial structure. Therefore, a wide range of applications of graphene have been developed including imaging, drug delivery, and biosensing. Among various newly developed graphene materials, the graphene quantum dots (GQDs) showed excellent fluorescence properties and preferable biocompatibility. It seems that the GQDs are becoming promising optical imaging reagents.

We have previously developed NIR-GQDs that demonstrated distinct in vitro and vivo imaging capacity. Based on this work, we have developed GQDs-doped mesoporous silica nanoparticles. The nanoparticles possess bi-function properties of NIR-imaging and drug delivery. Compared to the NIR-GQDs, the GQDs-doped silica nanoparticles retain the same near infrared fluorescence emission intensity. Additionally, owing to the pores in the MSNs could be modulated, the nanoparticles are promising carriers for drug delivery.
Trace analysis of explosive traces or residues in soil and post-blast debris for environmental and criminological purposes progressively gains importance and the need for sensitive and selective analysis of explosives in complex matrixes increases. In this study, a gold nanoparticle (AuNP) based colorimetric sensor that is easily applicable and capable of determination in picomolar level was developed for detecting trinitrotoluene (TNT) and 2,4,6-trinitrophenylmethylnitramine (tetryl). Detection principle of the sensor is a charge transfer interaction between the amine groups of compounds used to derivatize AuNP and NO\textsubscript{2}\textsuperscript{-} groups of TNT or tetryl on the surface of the modified AuNP. Spectroscopic evaluation for this proportional (ratiometric) sensor was made by taking the ratio of the absorption values of 650 nm to 520 nm. While the absorption value of 650 nm represents the new formed peak between 600-700 nm as a result of aggregation, the absorption value of 520 nm belongs to the original AuNP surface plasmons. Limit of detection (LOD) and limit of quantification (LOQ) for the developed sensor are LOD= 4x10\textsuperscript{-4} µg L\textsuperscript{-1} for TNT and LOD= 5x10\textsuperscript{-4} µg L\textsuperscript{-1} for tetryl. The sensor was applied to energetic material mixtures containing nitramine and nitrate esters. Additionally the interference effect of detergents, sugar, sweeteners, acetylsalicylic acid (aspirine), caffeine and paracetamol-based painkiller drugs, which are used as camouflage while carrying the explosives, were examined. This AuNP-based sensitive detection/quantification of TNT and tetryl is believed to be beneficial for in-field/on-site analysis of postblast debris in crime scene investigations and environmental sampling (such as TNT-contaminated land remediation).

**Keywords:** Forensic Chemistry, Molecular Spectroscopy, Nanotechnology, Sensors

**Application Code:** Nanotechnology

**Methodology Code:** Molecular Spectroscopy
Understanding the dynamics and conformation of subcellular structures have evolved rapidly in the last few years thanks to developments in high resolution imaging techniques. Imaging of soft tissues and cell imaging requires an extensive sample preparation as well as the use of state-of-the-art instrumentation. Our group and others have shown that through the use of micro-lenses in a conventional microscope a much higher spatial resolution (below 150 nm) can be achieved — an inexpensive and simple way for acquiring high-quality images in multi-modes. We have shown and demonstrated that by using a conventional inverted microscope and placing our liquid or solid micro-lenses between the objective and the sample, we are capable to resolve patterns in hard substrates in both the bright and the dark field as well as in fluorescence mode. We demonstrate here resolving biological and soft samples immersed in a liquid medium mimicking the fluidic and non-static environment of a cell.

Keywords: Fluorescence, Imaging, Microscopy
Application Code: Nanotechnology
Methodology Code: Microscopy
Fabrication of novel gold nanostructures is important in Surface Enhanced Raman Spectroscopy (SERS) since SERS amplification strongly depends on plasmons on the surface of nanoparticles. We report a novel synthesis of gold nanorods (AuNR) by seed-mediated growth procedure in the presence of benzalkonium chloride (BAC) surfactant. Our success relies on the introduction of the bromide–free surfactant BAC which is cheaper and less toxic than the most widely used surfactant cetiltrimethyl ammoniumbromide (CTAB). The effects of chemicals-reducing agent, stabilizing agent, gold, and silver were observed for homogenous and stable AuNR fabrication. It is observed that uniform AuNR were obtained in the growth solution and BAC was able to protect the AuNR over a year. BAC protected AuNR exhibited SERS activity similar to that of the CTAB protected AuNR. SERS effect of the AuNR were studied by comparing spectra of a Raman dye (DTNB) in the absence and presence of AuNR. BAC protected AuNR has almost 250-fold signal enhancement effect on DTNB and it is slightly higher than the signal enhancement effect of CTAB-AuNR on DTNB. Finally, we evaluated the SERS activity of the BAC protected AuNR in the quantitative analysis of potato virus X (PVX). A detection limit of 2.2 ng mL⁻¹ was achieved for PVX.

Keywords: Bioanalytical, Nanotechnology, Surfactants, Surface Enhanced Raman Spectroscopy
Application Code: Nanotechnology
Methodology Code: Vibrational Spectroscopy
Assessing the extent of release of both engineered nano-materials and associated capping agents (surfactants) from nanocomposite packaging materials into foods is a necessary part of evaluating their safety. Such exposure assessments rely on the availability of accurate methods to determine the amount of additives released from film packaging materials. Here we report on the development of a method to quickly and accurately measure the concentration of surfactants migrated from polymer films using flow-injection mass spectrometry (FIMS). We melt compounded low density polyethylene (LDPE) with surfactant-coated montmorillonite clays (Cloisite 20) and extruded the melt as free-standing thin films; films containing surfactant only were also produced. The surfactant used was Arquad 2HT-75, a quaternary ammonium compound derived from hydrogenated beef tallow. After extraction of the surfactant into environmental solvents, four quaternary ammonium ions were measured by FIMS: dioctadecyl-dimethylammonium, octadecyl-hexadecyl-dimethylammonium, dihexadecyl-dimethylammonium, and hexadecyl-tetradecyl-dimethylammonium. In this presentation we discuss the developed detection method, including instrument parameters, calibration standards, and validation techniques. We also present sample data associated with accelerated extraction experiments as well as migration experiments in food simulants for both surfactant-only and surfactant-clay elaborated test films.

Keywords: Food Safety, Liquid Chromatography/Mass Spectroscopy, Nanotechnology, Surfactants
Application Code: Nanotechnology
Methodology Code: Liquid Chromatography/Mass Spectrometry
Massive arrays of bipolar nanoelectrodes have been fabricated so as to permit spatial and temporal resolution of the exocytosis of oxidizable neurotransmitters (e.g., dopamine) in order to study the role of neurotransmitter release in neuronal communication. To this end, such arrays will be used to couple the oxidation of dopamine to a separate fluorogenic reduction reaction. This technique, referred to as fluorescence-enabled electrochemical microscopy, allows individual exocytosis events to be monitored and imaged with an optical signal, thereby providing a high degree of spatial resolution and eliminating the need for individual electrical connections with all of the electrodes in an array. The fabrication of these bipolar electrode arrays was carried out via laser interference lithography. In this process, a layer of photoresist is spin-coated onto a microscale silicon nitride window exposed in the surface of a silicon chip, after which the photoresist film was exposed to two interfering beams of light so as to create a mask for reactive ion etching of the underlying window. The final arrays were generated by thermally evaporating a gold film on the resulting nitride membrane such that individual electrodes were formed by gold exposed deposited within the holes etched in the nitride window. Note, however, that the individual electrodes comprising the arrays need not be electrically isolated from one another since electrical current follows the shortest possible path directly through the array and exhibits minimal lateral transfer between adjacent electrodes, an effect which is achieved by spacing the electrodes far apart relative to their respective thickness.

Keywords: Electrochemistry, Electrodes, Fluorescence, Imaging
Application Code: Nanotechnology
Methodology Code: Electrochemistry
Nanotechnology Applications

Tunable Plasmonic Nanostructures for Surface-Enhanced Raman Scattering

Surface-enhanced Raman scattering (SERS) is an emerging analytical technique used for characterization of biological and non-biological molecules and structures. Since plasmonic properties of the nanostructures is one of the most important factor influencing SERS activity, fabrication of tunable plasmonic properties of nanostructures are crucial in SERS studies. In this study, a novel approach to fabricate tunable plasmonic silver and gold nanodomes (AgNDs and AuNDs) based on combination of soft lithography and nanosphere lithography is presented. Convective-assembly method is used for deposition of latex particles on a glass slide uniformly and used as molds for polydimethylsiloxane (PDMS) to obtain nanovoid structures. The obtained nanovoids on the PDMS are used as template for fabrication of NDs. The nanovoids are filled with electrochemical deposition of Ag and Au to obtain metallic AgNDs and AuNDs respectively. Scanning electron microscopy (SEM) and atomic force microscopy (AFM) are used for the characterization of structural properties of the fabricated NDs. Optical characterization of the nanostructures is performed using SERS. The finite difference time domain (FDTD) method is used for calculation of plasmonic properties of the fabricated NDs. This study indicates that, the plasmonic properties of NDs are tuned not only by changing of the type of metal but also the diameters and heights of NDs.

Keywords: Surface Enhanced Raman Spectroscopy
Application Code: Nanotechnology
Methodology Code: Vibrational Spectroscopy
Enterovirus 71 (EV71) is one of the most common pathogens that cause hand-foot-and-mouth disease (HFMD) in humans. EV71 is likely to cause complications of nervous system in children. Thus, developing a reliable method for rapid screening of EV71 from other enterovirus infection is important. In this study, we develop a sandwich type enzyme-linked immunosorbent assay (ELISA) using EV71 antibody-modified gold nanoparticles (AbEV71\(\text{Au NPs}\)) for signal amplification coupled with cellulose acetate membrane (CAM) and laser desorption/ionization mass spectrometry (LDI-MS). The signal read out for EV71 concentration is directly proportional to the peak intensity of gold cluster ions ([Aun]\(^+\); \(n = 1\)\(\pm\)3) in the mass spectra. As the concentration of EV71 on CAM surface increases, higher number of AbEV71\(\text{Au NPs}\) are captured and therefore, the signal intensity of ([Aun]\(^+\); \(n = 1\)\(\pm\)3) increases. Each EV71 contains multiple sites for antigen-antibody interaction which allow signal amplification through AbEV71\(\text{Au NPs}\). Here, CAM is not only used to capture EV71 and separate impurities but also to effectively reduce background noise in the mass spectra. The AbEV71\(\text{Au NPs}\) based amplification system increases the sensitivity and thus achieves a limit of detection of about 1000 PFU/mL. This high-throughput LDI-MS detection of EV71 provides a reliable, low-cost and rapid method, and it may be employed for practical use such as virus screening during EV71 outbreaks.

**Keywords:** Immunoassay, Membrane, Nanotechnology

**Application Code:** Nanotechnology

**Methodology Code:** Mass Spectrometry
Recent research on exocytosis from PC12 cells has shown that only approximately 60% of the vesicular content is in fact released after stimulation. Partial release could potentially provide an ability to tune the strength of signaling and hence add a property of plasticity. In this project, we have investigated if the same phenomena occur in more complex systems as well. To answer this question, we have used [i]Drosophila[/i] larvae as a model system. Previously, exocytosis of octopamine from [i]Drosophila[/i] larvae type II varicosities had been measured by optogenetic stimulation.

The central nervous system is readily accessible in the body wall of [i]Drosophila[/i] larvae, and the octopamine containing type II varicosities can be visualized by genetically expressing a fluorescent label. As octopamine is electroactive, amperometry can be used as a quantification method with high temporal resolution. Nanotip electrodes have been carefully positioned inside a [i]Drosophila[/i] varicosity to carry out intracellular vesicle impact cytometry in these extremely small and challenging structures. In this method, intracellular vesicles impact, adsorb and then stochastically burst on the electrode. The current from oxidation of octopamine is used to calculate the total content of this transmitter in each vesicle.

By combining the developed technique for measuring total vesicular content with our more complex larva model, we calculate how many molecules are released per pore opening in complex exocytosis events. Our initial estimates suggest that a very small percentage of the vesicular content is released during exocytosis at neuronal varicosities.

Keywords: Bioanalytical, Electrochemistry, Neurochemistry, Quantitative

Application Code: Neurochemistry

Methodology Code: Electrochemistry
Surface-enhanced Raman spectroscopy (SERS) is one of the most commonly used techniques for explosive detection. Here we developed a highly sensitive, SERS based, self-assembled, flexible sensor for explosive detection by utilizing the strong electromagnetic enhancement of gold triangular nanoprisms. Our sensor is capable of detecting explosives (TNT, PETN, and RDX) at parts per trillion levels and has long-term stability and shelf life. Therefore, we believe that, this highly sensitive and highly selective SERS based sensor can be utilized for the identification or trace detection of explosives for investigation of national security concerns.

Keywords: Forensics, Nanotechnology, Sensors, Surface Enhanced Raman Spectroscopy

Application Code: Nanotechnology
Methodology Code: Sensors
The potential application of semiconductor nanocrystals in imaging and solid-state devices has facilitated not only various synthetic methods to produce monodispersed nanocrystals, but the investigation of how to better control their properties as well. The optoelectronic properties of these nanocrystals are controlled by the nature of the surface passivating ligands. However, existing growth models do not permit a complete understanding of the growth and formation process. These models rely solely on three stages (nucleation, diffusion growth, and ripening) of growth. Herein, we propose a new fourth stage describing the surface ligand attachment on to fully formed nanocrystal. This work investigated the existence of this step through time dependent spectrometry and spectroscopy characterization as well as through ex situ ligand attachment. Additionally, three different surface passivating ligands, with varying carbon chain lengths, were examined to determine how this parameter affects the growth mechanism. A complete understanding of the ligand adsorption process and the underlining kinetics will not only help expand the fundamental knowledge of nanocrystal growth and formation but also allow for the development of synthetic procedures to produce nanomaterials with unique optoelectronic properties to expand their applications.

Keywords: Energy, Mass Spectrometry, Material Science, Nanotechnology
Application Code: Nanotechnology
Methodology Code: Molecular Spectroscopy
Surface-enhanced Raman scattering (SERS) is an emerging analytical technique for characterization of biological and non-biological molecules and structures. Fabrication of tunable plasmonic nanostructures is the focal point for the design of novel SERS substrates due to their major contribution of electromagnetic enhancement to the SERS enhancement mechanism. In this study, a novel approach for the fabrication of Au-Ag, Au-Cu, and Ag-Cu alloy nanodomes having tunable plasmonic properties by changing of metal types and metal concentrations is demonstrated. First, convective-assembly method is used for deposition of the latex particles (1600 nm) on a glass slide uniformly to obtain template for the PDMS surface. After, PDMS is poured on the latex thin film to obtain nanovoids on the PDMS surface. The obtained nanovoids on PDMS are used as template for the fabrication of alloy nanodomes. The nanovoids are filled with the electrochemical co-deposition of two metals of interest to obtain alloy nanodomes having different metals and metals concentrations. The structural properties of all fabricated alloys are characterized using scanning electron microscopy (SEM) and atomic force microscopy (AFM). SERS is used for the optical characterization of the fabricated alloy nanodomes. The results demonstrate that, SERS performance of the alloy nanodomes is dependent both metal types and metals concentrations in the alloys.
Nanoscale electrochemical studies of nanoparticles (NP) can provide new insights into their structure-activity relationships. The use of small (5 nm radius) nanoelectrodes as tips in the scanning electrochemical microscope (SECM) recently allowed us to address individual 10-50 nm metal NPs attached to the flat substrate surface. In this paper, we introduce a new mode of the SECM operation based on tunneling between the tip and a nanoparticle immobilized on the insulating glass substrate. The obtained current vs. distance curves show the transition from the conventional feedback response to electron tunneling. In addition to high-resolution imaging of the NP topography, the tunneling mode enables measurement of the current flowing at a single NP without attaching it to the electrode surface. The developed methodology should be useful for studying the effects of nanoparticle size and geometry on electrocatalytic activity in real-world application environment.
Recent growth in commercial carbon nanotube (CNT) production resulted in a strong demand for analytical techniques capable of rapid characterization of these nanomaterials. Several techniques are currently being used for CNT characterization, including electron microscopy, Raman spectroscopy, thermo gravimetric analysis (TGA), and optical absorption spectroscopy. Due to the latest engineering breakthroughs in Raman instrumentation that dramatically improved analysis efficiency, portable Raman spectroscopy is becoming one of the most viable options for CNT characterization and/or quality control. First, this technique enables rapid and reliable detection of even small changes in CNT structure; second, it requires little or no sample preparation; third, portable Raman instruments are less expensive than the lab-grade analytical systems.

This poster compares performance of 532 and 785 nm Raman for CNT characterization, as well as presents a practical methodology to obtain CNT diameter distributions directly from Raman spectra. Even though both 532 and 785 nm Raman instruments are technically feasible for CNT characterization, our poster demonstrates several techno-economic advantages of 532 nm excitation. Raman signal at 532 nm is ~5-fold stronger than at 785 nm per unit laser power, therefore 532 nm portable Raman units are capable of ~5 times faster analysis and/or improved analysis quality. This advantage in speed and accuracy is combined with dramatically reduced instrument and analysis cost. Additionally, up to ~5 times reduced laser power can be used at 532 nm to eliminate laser-induced damage to gentle CNT samples without compromising analysis quality.

Keywords: Method Development, Nanotechnology, Quality Control, Raman Spectroscopy
Application Code: Nanotechnology
Methodology Code: Portable Instruments
One important property of metal NPs is their size-dependent electrochemical oxidation. Smaller size NPs also often show superior performance for catalytic and sensing applications due to high surface-to-volume ratio. A key aspect in the field is the synthesis of NPs with well controlled size and shape. In most cases, the size and shape are determined by fairly expensive and tedious electron microscopy methods, although electrochemistry have also been used. Our group recently showed that the oxidation potential of Au and Ag NPs decreases with decreasing NP radius. Linear Sweep Stripping Voltammetry (LSSV) can therefore serve as a potential method for metal NP size analysis. One drawback is that the oxidation potential also depends on the coverage, so it is necessary to keep the coverage constant or adjust for the coverage when comparing different sizes. Also, it is difficult to determine the size of larger NPs because the oxidation potential is not very different, due to the 1/radius dependence. In this work, we use a combination of cyclic voltammetry (CV) and LSSV to determine the size of Au NPs that is independent of the coverage and works for all size NPs. In CV, the oxidation and reduction cycle of Au in acidic solution allows an electrochemical measurement proportional to its surface area. In LSSV, complete dissolution of the Au by oxidation in bromide solution leads to an electrochemical measurement proportional to the entire volume of the NP. By knowing both the surface area and the volume, we can measure the surface-to-volume ratio (S/V), which is proportional to the NP radius in a mathematically predictable way. A plot of S/V versus 1/radius for 4 different sized Au NPs (radius = 2 nm, 6 nm, 10 nm, and 15 nm) is linear with a slope very close to the theoretical prediction of 3. This type of electrochemical analysis give a calibration curve that provides allows the simple determination of the NP size without an electron microscope or mass spectrometer.
Antibacterial materials play crucial roles in treating infectious disease caused by pathogenic bacteria. However, the rise of microbial drug resistance has become a problem due to the wide use of antibiotics and the emergency of antibiotic-resistant bacterial strains. Therefore, it is urgent to develop new generations of antimicrobial agents for effectively killing pathogenic bacteria.

In this work, we have successfully synthesized silver nanomaterials decorated reduced graphene oxide (rGO-Ag) as an antibacterial agent. A facile and green synthetic approach was developed to prepare Ag-rGO by using glucose and GO as a reducing agent and precursor, respectively. The synthesized nanocomposites were characterized by transmission electron microscopy (TEM), FT-IR, UV-Vis spectroscopy, Energy-dispersive X-ray spectroscopy (EDS). The data indicated that AgNPs have been successfully deposited on the surface of rGO. Moreover, we have also studied the effects of the amount of GO and AgNO3 on the density of AgNPs that deposited on rGO. The antibacterial activity of the synthesized rGO-Ag is currently under study. Our expectation is that rGO-Ag could be a promising antibacterial for clinical and environmental application.
New Methods

Acid Number of Crude Oils and Petroleum Products by Catalytic Thermometric Titration Using ASTM D8045

ASTM Standard D8045 describes the analysis of acidity in difficult crude oils and petroleum products using thermometric titration. This new technique overcomes solubility and precision challenges encountered with traditional potentiometric measurements. Thermometric titration is more precise, faster and reduces solvent requirements. Through eight years of development work in ASTM and thousands of sample measurements this new standard is shown to be rugged, robust and transportable across many labs and technicians. This poster provides a clear analysis of data acquired with this new method and details the benefits of thermometric titration for safer and more precise acidity analysis.

Keywords: Automation, Petrochemical, Titration
Application Code: Fuels, Energy and Petrochemical
Methodology Code: New Method
New Methods

Analysis of Ephedrine Alkaloids in Dietary Supplements Using a Fully Integrated GC/FT-IR/MS

Although dietary supplements containing ephedrine alkaloids have been prohibited by the FDA since 2004, the availability of such supplements persists. The ability to effectively and efficiently identify ephedrine alkaloids in these products is therefore an important role of the agency. Over the years, the FDA’s Forensic Chemistry Center has analyzed numerous dietary supplements containing ephedrine alkaloids. Ephedrine alkaloid diastereomers have similar electron ionization mass spectra, making it impossible to specifically identify them using only traditional GC-MS screening techniques. A targeted analysis method to identify each alkaloid has been developed, but it is time consuming and requires a two-step derivatization method that converts each alkaloid to the O-TMS, N-TFA derivative. In addition to being time consuming, selectivity is limited for this method because identification of each diastereomer is based solely on retention time. A simpler, faster and more selective method for differentiating ephedrine alkaloid diastereomers has been developed using gas chromatography with Fourier transform infrared and mass spectrometric detection (GC/FT-IR/MS). Supplements that were known to contain ephedrine alkaloids were used to establish the feasibility of the method. The performance of the infrared detector was compared to that of the mass selective detector, with respect to selectivity and sensitivity for underivatized, TMS and O-TMS, N-TFA preparations.

Keywords: Forensic Chemistry, FTIR, GC-MS
Application Code: Other
Methodology Code: New Method
Colorimetry is one of the effective on-site analysis for water quality because of useless analytical instruments. However, this method causes validation of analytical result because of depending on the intensity of human color vision. The validation of analytical result is improved by using portable type color analyzers, however, analytical cost is expensive because of instrument’s cost. The aim of our study is development of novel analyzer for water quality using smart devices (smartphone, phablet, and tablet). The proposed method is consisting of original attachment manufactured by 3 dimensional printer and original application software. Using our original attachment, sample cell is able to be fixed on image sensor of the smart device and screen light of the smart device act as light source. By using the system, colored sample is able to be taken a photo in same condition under various location and irradiation. By the use of original application software, the intensity of color is measured and date of image sensor is able to convert to concentration. As a result, performance of the proposed method was same as commercial portable color analyzer. Moreover, location and time date are obtained at the same time, and its results are also displayed on the map instantly. We investigated usability of the system in Japan and various countries. The results indicate that the proposed method not only reduce analytical cost but also provide new mapping function.

Keywords: Automation, Environmental, Environmental Analysis, Environmental/Water

Application Code: Environmental

Methodology Code: New Method
Aquatic plants play important roles in water environment. Except releasing oxygen and absorbing carbon dioxide, aquatic plants can remove or absorb excess nutrients and pollutants such as heavy metal ions in water. However, details about the removal or purification dynamics and mechanics have not been thoroughly understood yet. If the materials movements including DO transport across surface of any organs of the aquatic plants can be real-time and in situ monitored, it is definitely useful and helpful to understand the aquatic plants activities, including the removal or purification details. Here, Optical sensing of materials movements across a plant surface with a probe beam is reported. It is based on monitoring of deflection of a probe beam passing through a vicinity of the plant surface. The deflection of the probe beam was caused by concentration gradients of chemical species involved in the materials moments in physiological activities of the plant. Egeria densa was used as a model aquatic plant. The beam deflection at different locations of both leaves and stems in respiration process were monitored and compared. It is found that deflection signals changed with time and locations. Experimental results showed that the beam deflection method could be used for noninvasive sensing and monitoring of materials movements across the plant surface.

Keywords: Laser, Method Development, Monitoring
Application Code: Environmental
Methodology Code: New Method
We have developed a multichannel air displacement pipette with reconfigurable heads for non-standard liquid handling applications. Historically, multichannel pipettes have only been offered in linear configurations. These pipettes are designed for compatibility with well plates standardized for expensive, industrial liquid handling equipment. While linear multichannel pipettes enable many established assays, they do not support analytical tools with customized liquid holding geometries, specifically paper-based microfluidic devices. Using our pipette, complex paper-based microfluidic devices can be fabricated and tested without requiring i) multiple, time-consuming motions with a single-channel pipette or ii) device design limited to the configurations of traditional multichannel pipettes. With a simple and user-friendly design, this tool was created by modifying a commercial 12-channel pipette using machined and 3D-printed components. Our use of rapid prototyping equipment makes our inexpensive pipette readily adaptable for on-demand, device-specific applications. We demonstrate the quantitative capabilities of our pipette by performing calibration experiments, and the practical advantages of this tool are presented in the fabrication and use of a custom paper-based device. Our reconfigurable pipette supports the advancement of custom analytical tools with non-standard liquid handling requirements and provides an ergonomic alternative to commercial equipment for developers of these tools.

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Keywords:  Bioanalytical, Immunoassay, Lab-on-a-Chip/Microfluidics, Sample Handling/Automation
Application Code:  Bioanalytical
Methodology Code:  Microfluidics/Lab-on-a-Chip
Semiconductor manufacturing requires clean environments to minimize defect density and maintain cost effectiveness. It is essential then that the wafers are frequently cleaned throughout the many different fabrication stages. The use of dilute chemicals and ultrasonic stages has increased manufacturing yields by improving cleaning, process stability, and reliability. The improved cleaning efficiencies have improved yields to the point where the focus is now on improving the final drying step where the previous gains can be lost due to inefficient drying.

In the IPA vapor drying technique, sometimes referred to as Marangoni wafer drying, the wafer is gradually withdrawn from an aqueous bath into an atmosphere of IPA vapor and nitrogen. The IPA is absorbed into the water causing the meniscus to contract and causing the water to be removed from the wafer surface due to the gradient of surface tension.

Here we present the analysis of 6 semiconductor grade IPA samples for organic impurities that are typically not reported in the assay which is usually limited to the amount of metal and metalloids present. A Thermal desorption sampling technique is used to concentrate the volatiles in IPA prior to GCMS analysis to allow for excellent detection limits and impurity identification.

**Keywords:** GC-MS, Headspace, Semiconductor

**Application Code:** Process Analytical Chemistry

**Methodology Code:** Gas Chromatography/Mass Spectrometry
A discriminating dissolution method was developed to support a novel drug delivery system for a highly potent, low dose, and highly water soluble active pharmaceutical ingredient (API). The intended purpose of the method is to demonstrate extended release of a suspension formulation designed to treat pediatric populations. The delivery system consists of an anionic drug compound bound to a cationic resin which is then coated with a functional polymer. The drug is released from the resin via ionic exchange with a competing anionic molecule. Development of the dissolution method focused on optimizing parameters (e.g., media screening, sample size, pH) to assess an unconventional drug release from a coated, drug-loaded, ion-exchange resin. A reproducible and accurate dissolution method was developed that can distinguish between various formulation variables such as weight gain and coating composition. It was also discovered that in slightly basic dissolution media (pH 6.8-7.2), the drug released slowly (3-4 hrs) from the resin without any coating. Adding a functional coat onto the resin allowed for extended release up to 24 hours. Using the developed discriminating dissolution method, an ideal coating formulation can be identified to further aid in the development of an effective dosage form.
In the days of high speed high resolution digital data acquisition, it is hard to remember that peaks were once recorded fraction by fraction, point by point. Servo motors and chart recorders thankfully ended this but even then some of us will remember cutting out peaks from a chart or a copy thereof and weighing them for accurate quantitation. Digitization first entered the world of chromatography in the form of “integrators”, which allowed facile area based quantitation. Today available chromatographic software readily allows either height or area based quantitation. As long as one is in a domain where the detector response is linearly proportional to the analyte concentration in the detection cell, the peak area is a true representation of the amount of the analyte. Height is an approximation of the area and can represent area accurately only if the peak shape remains invariant. Both height and area based approaches to quantitation reduces two-dimensional chromatographic data to one, for a set of calibration data there can be only one height or area based calibration equation for quantitation. This approach has not changed in last 50 years. High speed high resolution data acquisition, however, also permits rapid computation of the width of a peak, at any number of chosen fixed heights (here we do not mean width at fixed fraction of the peak height as in width at half-height or width at 10% of the peak height, rather the width at specified signal values above the baseline, e.g., 0.1 volt, or 1 mAU or 1 μS/cm, etc.), leading to any number of applicable calibration curves. We will discuss in this presentation the many ways in which width-based quantitation outperforms height or area based quantitation.
The traditional approach to process monitoring is sending a production sample to the lab for testing. Unfortunately, the results often come back after it is too late to address any possible problems. Ideally, analytical results should be available while there is time to correct any issues. In this study, we demonstrate how some minor modifications to a benchtop GC can result in an automated, online instrument. We were able to monitor the off-gas from a fermentor in near real-time by adding a gas sampling valve to an benchtop GC and connecting the sampling valve to the off-gas port of the fermenter. This allowed the operator to monitor the progress of the fermentation as it was happening by simply pressing the GC start button to measure the ethanol concentration of the gas being produced. In addition, a custom chromatographic method was developed and optimized to provide results within one minute. The system was further automated by employing a sequence table to control the sampling valve. Not only can we measure the ethanol in the fermenter, but we can also monitor for signs of contamination, like acetic acid. In industrial fermentation facilities, contamination can be kept under control through the use of antibiotics if it is caught in time. Otherwise, the entire batch would need to be disposed of. This same technology can easily be adapted to provide real-time GC data to a variety of industrial processes.

Keywords: Automation, Biopharmaceutical, GC, Monitoring
Application Code: Process Analytical Chemistry
Methodology Code: Gas Chromatography
The use of marijuana and marijuana extracts for medicinal purposes has seen increased acceptance and approval in both North America and Europe. The chemical composition of marijuana is complex and, apart from cannabinoids, includes sugars, hydrocarbons, some proteins, fatty acids, phytosterols, terpenes, phenols, and flavonoids. This matrix complexity presents a challenge to laboratories faced with developing methods for the analysis of contaminants such as mycotoxins and pesticides. This presentation will present simple and effective sample preparation and analysis methodologies for three areas of interest in marijuana contaminant testing: pesticide residues, aflatoxins and residual solvents. Pesticides were analyzed by GC/MS/MS after QuEChERS extraction and cleanup using a new sorbent blend designed to remove pigment while increasing recovery of planar compounds. Aflatoxins were analyzed by LC/MS/MS after extraction and cleanup utilizing a solid phase extraction cartridge designed for cleanup of mycotoxin extracts. Residual solvents were analyzed from hemp extract using a simple headspace solid phase microextraction method followed by GC/MS. For all three applications, details of the methodologies will be presented along with data showing accuracies and reproducibilities for the analysis of spiked replicate samples.

**Keywords:** Chromatography, Food Contaminants, Sample Preparation, SPME

**Application Code:** Food Safety

**Methodology Code:** Chemical Methods
Medical cannabis refers to the use of cannabis and its corresponding cannabinoids, as a therapy to treat diseases and alleviate symptoms. Cannabis research has been severely restricting by strict laws and a DEA Class I scheduling of marijuana. Contrary to thousands of patients that already using medical marijuana as well as a growing number of manuscripts in peer reviewed, medical journals, cannabis research continue to be suppressed by government agencies. There are over 500 chemical moieties found in cannabis that cannot be found anywhere else in nature. This talk will examine some of these chemicals, as well as summarize many of these reported health benefits.

Cannabis quality control testing is critical for the accurate labeling of cannabinoids in medical cannabis products. Cannabis testing labs also measure terpene concentrations, as terpenes give cannabis its aroma and flavor and can provide an overall quality assessment of cultivation and handling. Cannabis testing labs also screen for many forms of contamination, including pesticides, metals, residual solvents, microorganisms, aflatoxins and mycotoxins. This presentation will examine the analytical instruments used for cannabis testing, as well as discuss the wide variety of cannabis products on the market. Mass spectrometry has emerged as a powerful tool for cannabis QC testing and research, and is particularly useful for the analysis of cannabis contaminants, including pesticides.

Lastly, we will look at analytical research technologies that may shed new light on the biochemical properties of cannabis and further aid in quality control testing. MALDI-TOF mass spectrometry is widely used in hospital microorganism identification, and can be applied to cannabis samples. MALDI-TOF MS also offers great opportunities for rapid cannabis strain typing. Online supercritical fluid extraction and supercritical chromatography (SFE-SFC), coupled with MS greatly simplifies sample preparation.

Keywords: Chromatography, Gas Chromatography/Mass Spectrometry, HPLC, Liquid Chromatography/Mass Spectrometry
This presentation will open up medical and anecdotal findings on the use of medicinal cannabis for many pediatric & adult diseases while success stories are shared & evaluated. With clinical research now being a reality, Tracy will discuss the path to research that will help lead us to finding the answers we seek for cancer, epilepsy, autism, Crohn's disease, PTSD and beyond.
Cannabis has been used as a medicine for thousands of years. We have only started to stigmatize this plant over the last century. It is known to help with inflammation, seizures and pain, as well as many other ailments. Recent developments have shown that cannabis treatments can be extremely beneficial in treating cancer, epilepsy, autism, chronic diseases, and many other conditions. We all have an endocannabinoid system in our bodies, but sadly most of us do not understand what is does for us. The endocannabinoid system as a mechanism for drug interaction is widely unexplored, and the true potential for positive impact on human health is unknown.

This presentation will provide a physician’s view on medical cannabis, delivered by a Cannabis Therapeutics Specialist trained in Family Medicine, Occupational & Environmental Medicine. Drawing from over 30+ years of experience in both research and patient care, this presentation will highlight the need for better therapies and a new focus on total health care (www.TotalHealthCareTHC.com). It is time to move beyond the misconceptions and misinformation associated with this healing plant. It is time to increase education and reduce incarceration, and to learn more about the healing properties of cannabinoids and other medicines in cannabis. Let’s move beyond the stigmas associated with THC towards total health care.

Keywords: Biomedical, Clinical/Toxicology, Medical, Natural Products
Application Code: Other
Methodology Code: Education/Teaching
Atomic Spectroscopy Instrumentation Development: A Disconnect Between the Research Laboratories and Academic Research to Marketplace: Can a Better Link be Forged?

The field of analytical atomic spectrometry, like most others, depends in part on instrumental innovation. Some of that innovation derives from research in the private sector, although private investment in research has been in decline. Other research, carried out in universities and government laboratories, seems exciting or appealing, but often never finds its way into the marketplace. The reasons for this disconnect are worth considering. Is it that academic or fundamental research is too far removed from practical needs? Do instrument firms suffer from the “Not Invented Here (NIH)” syndrome? Are vendors concerned about undermining their own existing products? Are market needs already satisfied? The answers to these and other questions will be answered in part by examples from the speaker’s personal experiences. Two examples of successfully marketed instruments and two examples of unsuccessful ventures will be provided; differences will reveal that the above factors play a role, but that early establishment of interaction between an instrument firm and a university or government laboratory goes a long way to ensuring successful technology transfer.

**Keywords:** Atomic Absorption, Atomic Spectroscopy, Instrumentation, Mass Spectrometry

**Application Code:** Other

**Methodology Code:** Atomic Spectroscopy/Elemental Analysis
The relationship between University (and National Labs) and Commercial Organizations (PerkinElmer in my case) has been “interesting” to say the least. In my early days with the Company (beginning in 1982) we have had, at times, extensive collaborations with Universities. At that time, most were involved with developments related to Graphite furnace and AAS developments. I believe these relationships were regarded as fruitful. In the mid-to-late 80’s these interactions had run their course and gradually ended.

In the late 90’s our efforts were very much focused on the ICP and ICP-MS developments. We maintained a few relationships with academic, national labs and other external resources but there was not broad outreach to form collaborations.

In recent years, there has been a renewed interest in developing and maintaining collaborations with academic research groups as a way to find and advance useful technologies.

In this presentation, I will share thoughts on where I believe the various facets of atomic spectroscopy are headed and how collaborations can be beneficial to both parties. I will also share some thoughts in how “good” collaborations should work.

**Key Words:** Elemental Analysis, ICP, ICP-MS

**Application Code:** General Interest

**Methodology Code:** Atomic Spectroscopy/Elemental Analysis
The development and application of gas-phase ion molecule chemistry to reduce interferences in ICPMS represents a success story in working with the analytical instrumentation industry and in effecting technology transfer that changed the face of ICPMS, vanquishing what to then had been considered the Achilles Heel of ICPMS. This paper will review the history and development of this approach, covering early inspirational work and describing development work conducted at PNNL to help effect this transformation in ICPMS. Other examples, both successful and not, will also be discussed in an effort to illustrate the trials and tribulations in commercializing new analytical approaches and technologies.

Keywords: Atomic Spectroscopy, ICP-MS
Application Code: Environmental
Methodology Code: Atomic Spectroscopy/Elemental Analysis
Research in this laboratory over the last three decades is best classified as instrumentation development. The majority of those efforts have centered on the use of low-power glow discharge (GD) sources for spectrochemical analysis by optical and mass spectrometries (OES/MS). Early work sought to extend the utility of low-pressure GD sources, which heretofore had been powered by direct current potentials, to radio frequency (rf) powering. This technology (the subject of 4 patents) opened up the field of direct solids analysis to include bulk insulators and non-conductive coatings. These efforts set the basis for what is now the norm in GD-OES, and were funded by the National Science Foundation (6 yrs), Extrel (2 yrs), VG/Fisons (4 yrs), and, Jobin-Yvon (10 yrs). Interestingly, the Extrel support went to the construction of a double-quadrupole mass spectrometer, which is now the norm in ICP-MS. Since the early 2000s, a great deal of world-wide research has been devoted to the development of small platform plasma sources as low cost, perhaps transportable alternatives to ICP sources, particularly for OES. This laboratory has developed the liquid sampling-atmospheric pressure glow discharge (LS-APGD) microplasma. Unique among all of the other liquid electrode GD sources, the LS-APGD is also operable as an MS ionization source – both for elemental and molecular species analysis. Also unique, samples can be introduced in the solution, particulate (via laser ablation), and gas phases. It has been demonstrated that elemental and molecular species analysis can be affected through an ambient desorption process. Finally, it has been shown that the device, when coupled to an Orbitrap mass analyzer, can deliver high sensitivity, accurate, and precise isotope ratio data. To date, the LS-APGD developments have been supported by a number of US government programs, but as of yet, not received appreciable commercial interest. Neither have the other sources. Why not?
Mass Cytometry is a specialized enactment of Inductively Coupled Plasma Mass Spectrometry configured for the analysis of multiple biomarkers in single cells and in subcellular imaging of tissues. This will be a story about the interaction (or lack thereof) of industry, university, government and arms'-length government agencies, investors and, most importantly, a talented and committed team of inventors, scientists, engineers, technicians and commercial champions.

Conceived at and initially supported by MDS Sciex, a funding application to the Canadian funding agency Genome Canada received the unusual review that the proposal was “not likely to succeed but worth the risk”. Unable to secure the necessary co-funding from their employer and potential corporate partners, the inventors moved to the University of Toronto where they prosecuted the development to the alpha prototype stage with the support of a variety of government (US and Canada), institutional and corporate contributions. While pursuing (and failing to obtain) venture capital in Canada, the technology came to the attention of Gary Nolan at Stanford, Mario Roederer at the NIH and Susanne Heck of Guys’ and St. Thomas’ Hospital, London. Their enthusiasm ultimately attracted successful venture capital in the US and Europe, enabling the maturation of DVS Sciences with an R&D and manufacturing facility in Canada and a global commercial headquarters in California. Three years of exceptional achievements in engineering, manufacturing and commercial success inspired the investors to pursue an exit through acquisition. Fluidigm Inc., an innovative single-cell genomics company based in California, saw the complementary opportunity in single-cell proteomics, and the companies merged in 2014.

The story will highlight the cooperation and commitment of the technical team and the courage and contributions of institutional support that bridged the early-stage company chasm.

Keywords: Atomic Spectroscopy, Biotechnology, Elemental Analysis, ICP-MS
Application Code: Bioanalytical
Methodology Code: Atomic Spectroscopy/Elemental Analysis
Vesicular exocytosis is an essential and ubiquitous process in neurons and endocrine cells by which neurotransmitters are released in synaptic clefts or extracellular fluids. It involves the fusion of a vesicle loaded with chemical messengers to the cell membrane, a stage conventionally thought to be followed in endocrine cells by the full integration of the vesicle membrane into the cell one.

We will show how precise reconstructions of the fusion pore expansion kinetics together with its maximal opening can be derived from amperometric currents time-courses. These data definitively establish that under normal conditions (wild cells in physiological medium), fusion pores may enlarge at most to ca. one fifth of the radius of their parent vesicle, hence ruling out the “full fusion” conservative paradigm.

A second part of the talk will be devoted to the discussion of the origin of amperometric spikes that display decay branches described by a two-exponential kinetics. Indeed, recent data from the Ewing’s group have established that these events represent a significant fraction of those recorded in amperometric traces.
Dopamine fluctuations occur on two timescales; rapid, sub-second (phasic) firing and slower, minute-to-minute (tonic) changes. Fast-scan cyclic voltammetry (FSCV) has long been used to study phasic dopamine transmission. However, this technique requires background subtraction. Thus baseline levels, and with them tonic changes, are inaccessible. Fast-scan controlled adsorption voltammetry (FSCAV), a modification of FSCV, provides access to these tonic concentrations with sub-minute temporal resolution. Sub-anesthetic ketamine has recently been investigated as a therapeutic for depression and to reduce L-DOPA-induced dyskinesias resultant from long-term Parkinson’s disease (PD) treatment. Here, we use FSCAV to monitor fluctuations in tonic dopamine at chronically implanted carbon-fiber microelectrodes in the dorsolateral striatum (terminal region of substantia nigra dopaminergic neurons highly degraded in PD) of freely moving rats at thirty-second intervals. Measurements were made across several weeks and in response to ketamine injection. Two three-hour no-injection control sessions were recorded and stable tonic levels were observed ([DA]tonic = 250 ± 20 nM). In separate sessions, either vehicle or sub-anesthetic ketamine (20 mg/kg) was administered intraperitoneal after one hour of baseline measurement. Preliminary data suggests saline administration does not affect tonic dopamine while ketamine injection elicits a rapid (timescale) reduction in concentration which recovers within ~30 min.
In Vivo Neurochemistry: Applications from Single Cells to Behavior

Advances in Microdialysis with LC-MS to Determine Chemistry Underlying Behavior

Microdialysis sampling coupled to LC has been used for over 30 years to measure neurochemicals in the brain of live subjects. Most studies have focused on pharmacology, i.e., determining how specific small molecule neurotransmitters are altered during drug administration. In this work we demonstrate how LC-MS can be used to measure many neurotransmitters and their metabolites. The techniques are combined with rapid sampling to monitor spontaneous neurochemical changes with behavior and changes evoked by selective neuron stimulation, e.g. optogenetic. These successes are also made possible by high sensitivity methods and novel sampling probes. Finally, new probes based on microfabrication are enabling higher spatial resolution for in vivo studies.

Keywords: Derivatization, Liquid Chromatography/Mass Spectroscopy, Neurochemistry

Application Code: Neurochemistry

Methodology Code: Liquid Chromatography/Mass Spectrometry
Dysfunctions 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 utilize novel voltammetric tools and mathematical models to characterize serotonin neurotransmission in different animal models of depression. Specifically we compare behavioral depression to depression due to toxin exposure, obesity and neurodegeneration. We find that, although animals all exhibit the same behavioral symptoms of depression, the serotonin system is affected in different ways by different depression etiologies. We model the experimental data and offer mechanistic insights into how different extracellular mechanisms that regulate serotonin circumstantially dysfunction. This leads us to hypothesize why antidepressants are not universally effective.
In the brain ‘reward’ system, the neurotransmitter dopamine is believed to play a key role in mediating goal-directed behaviors for ‘natural’ reinforcers (e.g., food and water), as well as abused substances such as cocaine. However, examining the precise role of dopamine in reward processing requires the ability to measure rapid dopamine signaling in ‘real time’ in behaving animals. Indeed, the advancement of the neurochemical method, fast scan cyclic voltammetry, to awake and behaving rats championed by Mark Wightman enabled detailed investigations of the role of dopamine in reward. Here, I will highlight some of our 17-year collaborative research on this topic. Critically, this research would not have been possible without the ability to combine electrochemical methods in awake, freely moving animals with sophisticated behavioral tasks. While mesolimbic dopamine is often attributed to simple ‘reward’ processing, our work has shown that rapid dopamine signaling plays a key role in more complex aspects of neural processing including associative learning, decision making and drug addiction.
The mixtures of molecules found in exhaled human breath are derived from the human exposome and from normal and abnormal physiology. In general, the concentrations of endogenously produced molecules are lower in breath than the corresponding concentrations of molecules from exogenous sources. Unique molecules in breath originate from the ingestion, inhalation, or dermal absorption of exogenous substances or from the metabolism of foreign cells (bacteria, viruses, or eukaryotic organisms). Many of these foreign cells are resident in the gut or respiratory tract.

Normal cellular biochemistry can only be induced or suppressed by abnormal physiology and although disease states may appear to be producing unique molecules these results are only a reflection of the detection limit of the analytical method.

Breath biomarker discovery is a complex task and molecules that may be present in breath as a result of exposure to exogenous sources must be identified. Differentiating between normal and disease states may be made when a particular breath molecule exceeds or is depressed below a given concentration or when the profiles of the concentrations of a group of molecules differ from concentration profiles of breath collected from healthy people.

Clearly the use of breath for diagnosing disease will require that the concentration profiles of breath molecules for normal healthy human subjects be established and these studies should include such variables as age, gender, ethnicity, body mass index and personal habits. This talk will discuss critical issues in clinical breath analysis.
Metabolomics: Breath as a Sample for Clinical Analysis

Breath Metabolomics in Lung and Systemic Disease

Our breath includes substances we produce endogenously as part of our normal (or disease-related) metabolism whether this is local in the lung or systemic in origin. Since we are constantly inhaling air from our environment as we breathe in the ambient air, exhaled breath can also reflect our environmental exposure(s). Furthermore, our breath contains volatile compounds produced by our “internal environment”: the bacteria in our gut and mouth. Add to all of those volatile byproducts generated from our diet, medications, drugs, or toxins that we are exposed to and you get a very rich matrix that has great potential to revolutionize and personalize medicine. Sensor array (electronic nose) devices can be trained to recognize patterns or “smell-prints” but this technology is not well-suited to identify specific compounds. Mass spectrometry devices on the other hand, allow identification of specific individual compounds in the breath, but are not well-suited to recognize patterns commonly seen in disease. We have used an approach that combines the strengths of both methods. By approaching each compound (or peak) on the mass spectrometry output as its own sensor, we are able to recognize patterns or “breath-prints” in mass spectrometry data in a way similar to how the sensor arrays recognize “smell-prints”. Unlike pattern recognition by the sensor array based systems, the major strength of our approach is that we are able to identify the single components that contribute to each pattern we recognize. With this best-of-both-worlds approach, we are able to identify unique “breath-prints” in patients with liver disease (fetor hepaticus) as well as heart and kidney disease. We are further able to analyze these patterns to identify single molecules in the breath of these patients and link them to the underlying pathobiology of the disease.

Keywords: Biological Samples, Biomedical, Clinical/Toxicology, Data Analysis
Application Code: Biomedical
Methodology Code: Mass Spectrometry
The experimental challenges presented by the analysis of volatile organic compounds (VOCs) in exhaled breath intended to identify reliable biomarkers are highlighted. It is stressed that positive identification and accurate quantification of the VOCs are imperative if they are to be adopted as discreet biomarkers. Breath sampling procedures are discussed and real-time sampling and analysis is desirable for accurate quantification. Whilst recognizing such real-time analysis is challenging and sample collection is often required because of low trace compound concentrations, objective recognition of the pitfalls involved in this is essential. It is emphasized that mouth-exhaled breath must be partially contaminated by orally generated compounds and so, when possible, analysis of nose-exhaled breath should be performed. Some difficulties in breath analysis are mitigated by the judicious choice of analytical instrumentation, but no single instrument can provide solutions to all the analytical challenges. Analysis and interpretation of breath analysis data, however acquired, needs to be treated circumspectly. In particular, the excessive use of statistics to treat poorly described mass spectrometry/mobility spectra should be avoided, since it can result in unjustifiable conclusions. It is should be understood that recognition of combinations of VOCs in breath that, for example, apparently correlate with particular cancer states, will not be taken seriously until they are replicated in other laboratories and clinics. Finally, the inhibiting notion that single biomarkers of infection and disease are unlikely to be identified and utilized clinically should be dispelled by the exemplary and widely used single biomarkers NO and H2 and now, as indicated by recent selected ion flow tube mass spectroscopy (SIFT–MS) results, hydrogen cyanide, pentane and acetic acid. Hopefully, these discoveries will stimulate researchers to be more open-minded on this important issue in medicine.

Keywords: Analysis, On-line, Trace Analysis, Volatile Organic Compounds
Application Code: Genomics, Proteomics and Other 'Omics
Methodology Code: Mass Spectrometry
According to recent research, 70-90% of long-term latency and chronic human disease incidence is attributable to environmental (human exposome) factors through the gene - environment interaction. Environmental and clinical sciences are now embarking on a new “discovery” path for decoding the human exposome using biomarkers in breath and other related biological media. In addition to gas-phase organics, the exhaled breath also contains aerosols that carry proteins, cell fragments, bacteria and DNA. Current research is exploiting advances in GC-MS and LC-MS as well as immunochemistry, to measure as many analytes as possible in breath and breath condensate without preconception. By compositing results into case-control groups, differences can be attributed to the previous exposure or the pre-clinical disease state. Results of such agnostic discovery analyses can then be translated to targeted approaches to streamline diagnoses. We describe how these methods will be used in our laboratories to explore firefighters exposures and cellular respiration in bioreactors.
Since its inception, field asymmetric ion mobility spectrometry (FAIMS) has been envisioned as a field-portable device, as it affords less expense and greater simplicity than many similar methods. Portable FAIMS could provide in situ analysis, allowing researchers to bring the lab to the sample. Ideally, these are simple, robust devices that may be operated by non-professional personnel, yet still provide adequate data when in the field. While reducing the size and complexity tends to bring with it a loss of performance and accuracy, this is made up for by the incredibly high throughput and overall portability of the instrument. Moreover, the point-of-care (POC) FAIMS device can be brought back to the lab and coupled to a conventional mass spectrometer to provide the necessary method development and compound validation. However, to realize such a device, several considerations for miniaturization of the instrumentation must be discussed, as well as how the future of various applications may benefit from the development and acceptance of POC FAIMS.

In preliminary studies, numerous standards were analyzed by both home-built and commercially available FAIMS systems. Several components necessary for a miniaturized POC FAIMS device are currently under development; notably, a novel asymmetric square-waveform generator that is responsible for separation of ions by FAIMS, and a standalone detector which allows the FAIMS device to operate without the need for a mass spectrometer. These components were tested for function, efficiency, and reliability, and the data will be presented here.

Keywords: Biomedical, Biotechnology, Detection, Instrumentation
Application Code: Biomedical
Methodology Code: Portable Instruments
Electrospray ionization (ESI) with mass spectrometry (MS) is widely used for protein structural characterization and identification. Native MS, where ions are formed from buffered aqueous solutions in which proteins have native structures and reactivities, enables large protein and other macromolecular complexes to be transferred intact from solution into the gas phase. Information about stoichiometries and structure can be obtained from such measurements. A key question remains about what elements of solution-phase structure are retained in the gas phase. Here, methods to manipulate protein structure in the electrospray droplet before ion formation will be discussed. Supercharging methods can significantly increase the extent of ion charging in native mass spectrometry; the origin of the supercharging effects will be discussed. A novel method to perform rapid mixing immediately prior to ESI droplet formation using theta glass capillaries will be presented. With this method, proteins can be induced to either unfold or to fold in the ESI droplets, and the reaction time can be readily varied by changing tip diameters. This method can be used to investigate protein folding that occurs faster than 1 μs, which is an order of magnitude faster than is possible with conventional mixing experiments and uses ~10,000 times less material.
Aggregation of peptides and proteins is a root cause for many devastating diseases including Alzheimer’s, Parkinson’s and Type 2 Diabetes. Recent evidence implicates early oligomer states as the primary toxic agents in these diseases. Structural and mechanistic information is very difficult to obtain on these systems using traditional analytical methods due to the complex nature of the reacting solutions. Here we will present data on assembly of a number of peptides and amino acids using a combination of ion mobility based mass spectrometry, ultra high resolution atomic force microscopy and infrared spectroscopy of size and shape selected oligomers. Structural information will be reported along with details on the mechanism of the assembly process. Implications for application to a number of important diseases will also be made.

Keywords: Amino Acids, Bioanalytical, Mass Spectrometry, Peptides
Application Code: Bioanalytical
Methodology Code: Mass Spectrometry
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. Molecular images are produced in specific m/z (mass-to-charge) values, or ranges of values. Each specimen produces hundreds of specific molecular images from a single raster of the tissue. In a complementary approach, histology-directed imaging, mass spectra are collected from selected areas of cells for laser ablation and analysis. We use IMS in biological and medical research projects such as diabetes, embryo implantation in mouse, assessment of margins in renal cancers, macular degeneration, and neurodegenerative disease. Molecular signatures are identified that are differentially expressed in diseased tissue compared to normal tissue and in differentiating different stages of disease. These signatures typically consist of 8 - 10 or more different proteins and peptides, each identified using classical proteomics methods. One application is the differentiation of benign skin lesions from melanomas using PIMS (Pathology Interface for Mass Spectrometry) developed in-house. IMS has been applied to drug targeting and metabolic studies in organs and intact whole animal sections. I will describe advances 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 just a few minutes. Applications include the use of MS/MS, ultra-high mass resolution, and ion accumulation devices. New biocomputational approaches 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 are discussed.
Mass spectrometry (MS) is a technique that weighs molecules, but this simple measurement can reveal much more than size. MS has capabilities to offer structural biologists layers of insight into the details of protein complexes. Using electrospray ionization (ESI), mass measurements deliver information on stoichiometry of binding partners directly. The MS analysis of biomolecules from non-denaturing solution conditions is now called “native” MS. Relative charging by native ESI-MS can give some information on protein folding. Top-down mass spectrometry is an effective tool for protein sequencing. We use high resolution Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) to generate topological information for large proteins and complexes. We are using electron capture dissociation (ECD) and other means for activation/dissociation, including infrared multiphoton dissociation (IRMPD), ultraviolet photodissociation (UVPD), and electron ionization dissociation (EID) to generate complementary structural information. Solubilizing membrane proteins in MS-compatible detergents or lipid structures are effective for delivery into the mass spectrometer, but require efficient means to unwrap the native membrane protein from their cocoon for detection. We show that FT mass spectrometers can be especially effective for “shaking off” detergents and lipids from native membrane proteins for subsequent MS and top-down MS measurements. The importance of salt bridges in the dissociation behavior of gas phase proteins will be presented. We aim to relate the 3D architecture of the gas phase protein to the solution phase state as a means to further develop MS for structural biology.

Keywords: Bioanalytical, Electrospray, Mass Spectrometry, Protein
Application Code: Bioanalytical
Methodology Code: Mass Spectrometry
Proteins and their complexes are dynamic entities. Indeed, co-existing, transiently populated conformations play crucial roles in some of the fastest growing major diseases in the US including Alzheimer's disease, Type 2 Diabetes, atherosclerosis, or cancer. However, detailed structures of proteins and their complexes implicated in these disease are often not directly amenable with available biophysical methods that provide time- and/or ensemble-averaged structures.

Ion mobility spectrometry-mass spectrometry (IMS-MS) is capable to reveal such transient structures, but still suffers from two major shortcomings. First, IMS-MS is a gas-phase method. Hence, it is often unclear how relevant IMS-MS data actually are for understanding biological processes that occur in solution under physiological conditions. Second, IMS-MS measures an orientation-averaged cross section of a protein. It is thus challenging to extract detailed protein structures from only IMS-MS data. However, exactly this is key for developing pharmacological strategies to treat or prevent diseases.

In this talk, we discuss advances made in our lab towards using IMS-MS data in an automated manner to determine solution structures of proteins de novo, i.e. from IMS-MS data alone. The central aspects of our work is to integrate experimental and computational approaches for time-resolved IMS-MS that were recently developed in our lab.
There is an increasing trend towards miniaturization of biological and chemical sensors, and their integration with miniaturized sample pre-processing and analysis systems. Because of its simplicity and the required instrumentation is fully portable, electrochemical transduction has received great recognition over other mechanisms and has been widely used in numerous low-cost devices. Particularly, amperometric transducers are frequently applied on screen-printed electrodes (SPEs) based sensors for the analysis of pharmaceutical compounds. SPEs are disposable planar devices, which can be produced in large scale over different substrates and designed according to the application.

Several electrochemical systems based on the use of SPEs for the quantification of pharmaceutical compounds are presented: acetaminophen, triptans, phenylephrine, dopamine and catecholamines.

An amperometric detection of acetaminophen is carried out using two different setups: a thin layer flow cell SPEs integrated in a miniaturized flow system and SPEs coupled to a batch injection analysis cell (BIA). The two devices are used to determine the acetaminophen content in commercial formulations.

Based on their irreversible oxidation processes, two voltammetric methods have been optimized to quantify both triptans and phenylephrine using small volumes of sample. The latest one is applied to monitor the stability of the drug in different store conditions in an easy and rapid procedure.

Nanostructured SPEs are used in the electrochemical method developed to quantify dopamine, focusing in the different electrochemical behaviour of this compound in presence of graphene nanomaterials, allowing us to discriminate between different compounds without any separation steps.

This is not the case with detection of catecholamines where an HPLC system is required to resolve the electrochemical simultaneous determination in urine samples.

Keywords: Drugs, Pharmaceutical, Portable Instruments, Voltammetry
Application Code: Pharmaceutical
Methodology Code: Electrochemistry
Pharmaceutical Applications of Electrochemistry

Optogenetics and Biosensing Applications

Abstract Text

Optogenetics harnesses a combination of genetic and optical techniques to directly control specific neural circuits. Biosensors measure specific analytes with 1-second temporal resolution in real-time with minimal perturbation to the surrounding tissue. Optogenetics, biosensors and the existing pharmacological toolset for awake, freely moving animals empower techniques in which the function of complex neural circuitry can be investigated by precisely altering the state of, and monitoring, neurophysiological systems. In this presentation, we will describe in vivo measurements of lactate, glucose, and glutamate with sleep/wake state to emphasize the importance of knowing the normal variation in neurotransmitter levels for a particular analyte before initiating an experimental protocol. We will also describe direct optogenetic stimulation of the hippocampus in transgenic rodents and the resulting seizure activity measured both with cortical/depth EEG and biosensors. Mice were stimulated in the hippocampus for 20 s with blue (445 nm) or deep red (660 nm) light at 20 Hz, 10% duty cycle, (intensity > 100 mW/mm²) while measuring EEG via a depth electrode in the hippocampus and a cortical electrode in each hemisphere. Moreover, in a subset of mice, the same experiment was performed with the addition of a glutamate biosensor implanted in the prefrontal cortex. In response to blue light stimulation, all transgenic mice had EEG frequency changes in the hippocampus corresponding to the timing of the stimulation pulse. In 70% of the trials, the response propagated to both hemispheres resulting in large-amplitude, cortical seizure-like, activity lasting 20 – 30 s after stimulation activity. Glutamate concentration consistently changed with stimulation. As an additional control, stimulation at 660 nm (deep red) did not result in any measurable response in either EEG or extracellular glutamate concentration.

Abstract Text

Keywords: Biosensors, Electrochemistry, Neurochemistry, Optogenetics

Application Code: Neurochemistry

Methodology Code: Integrated Sensor Systems
Pharmaceutical Applications of Electrochemistry

Electrochemistry for Detection, Reaction and Synthesis in Pharmaceutical Research

Electroanalytical techniques have found their way to many applications in various fields of research. In this presentation attention will be paid to electrochemical detection (ECD) in HPLC, and to electrochemical reactors for synthesis of biologically interesting products. Although these are two totally different topics, the common denominator is the equipment used, and the company producing it....

Electrochemical detection in HPLC is being used in several research fields such as pharma, clinical and neuroscience analysis. The QC testing of drug compounds is performed on a worldwide scale by pharmaceutical companies, contract research labs and various other institutions. Regulatory requirements are broader and more stringent than ever before and will only increase to protect human and nature. A number of HPLC/ECD applications will be presented of United States and European Pharmacopeia methods, for quality control of pharmaceutical products such as aminoglycoside antibiotics, fluorodeoxyglucose, heparin and Betadex sulfobutyl ether sodium.

Electrochemical reactors are applied for synthesis of metabolites of pharmaceutical products. In combination with mass spectrometric detection it creates a powerful platform to investigate various oxidation and reduction processes in life sciences. It is a complementary technique to traditional in vivo or in vitro metabolism studies, and delivers the oxidative metabolic fingerprint of a molecule in a very short time. Recently, the use of EC/MS has been extended towards pharmaceutical stability testing and environmental degradation. Also in the field of top down proteomics electrochemical reactors have been applied successfully to reduce disulfide bonds of proteins prior to MS analysis. This method was successfully applied for characterization of proteins by hydrogen/deuterium exchange monitored by mass spectrometry (HDXMS).

Keywords: Biopharmaceutical, Characterization, Electrochemistry, Liquid Chromatography/Mass Spectroscopy
Application Code: Pharmaceutical
Methodology Code: Liquid Chromatography/Mass Spectrometry
Pharmaceutical Applications of Electrochemistry

Development of Electrochemical Paper-Based Devices for Diagnostics and Preventive Care

High-technology was critical to push progress in diagnostics, monitoring of patients, drug discovery and biological and chemical analysis. The past decades also saw a large effort towards low-cost diagnostics tools for point-of-care (POC) due the cost, maintenance and infrastructure associated with it preventing a global access to those hi-tech tools. Light-weight and global available, paper is a good candidate for portable, low-cost and simple platforms that permit efficient and convenient analysis at the POC. Although colorimetry is frequently used, we will focus on electrochemical devices which have the additional advantages to permit quantitative analysis and are not susceptible to color or particulates interferences in samples. A drawback of electrochemistry for POC was the need of a potentiostat, however examples showed that inexpensive commercial readers, or even cellular phone can be used. The Electrochemical Microfluidic Paper-based Analytical Devices using a commercial glucometer as a reader demonstrated their potential to quantify analytes such as glucose, lactate and cholesterol. Electrolytes imbalances measured in blood or sweat are also useful for health assessment as well as nutrition quality control. Ion-selective electrodes usually utilized can be fragile, costly, and subject to biofouling. We are generating micronutrients sensing platforms based on voltammetric solid-state ion-sensing, using potassium as first target. Paper can also support culture of bacteria or 3D construct of cancer cells for compounds testing. By combining culture capabilities and electrochemistry, we are developing electrochemical paper-based devices for the culture and detection of bacteria. These various examples will highlight how paper and electrochemistry combined advantages to bring new tools for pharmaceutical and analytical applications.

Keywords: Bioanalytical, Biological Samples, Electrochemistry, Material Science
Application Code: Bioanalytical
Methodology Code: Electrochemistry
A major challenge in utilizing infrared (IR) spectroscopic imaging for biomedical uses has been to develop the technology for clinical applications. Here we discuss the range of scientific advances – from theory to statistical analysis of results – that need to made for a successful protocol. We describe recent progress in the area, focusing especially on the development and use of high definition (HD) IR imaging. Concomitant with emerging new capabilities and instruments are challenges arising from massive data sizes, subcellular diversity in spectral signals, effectively utilizing selection of parameters for optical design and confidence in results. Here we first discuss theoretical approaches to system design and performance of IR imaging systems. High performance microscopes are used for matching image quality for specific uses, and imaging for pathology obtained. Next, we provide a description and challenges of various histologic models and report on fast imaging for digital molecular histopathology. Finally, we describe combined spatial-spectral imaging with quantitative estimates of the accuracy of results. Together, these examples provide new insight into design of instruments, selection of parameters, evaluation of tissue imaging protocols and quantification of confidence in results for pathology.

Keywords: Biomedical, FTIR, Imaging, Microscopy
Application Code: Biomedical
Methodology Code: Vibrational Spectroscopy
Transplantation remains the main treatment for end-stage organ failure. Due to the extreme demand for organs for transplantation it is critical that transplanted patients are closely monitored for complications to ensure that grafts remain viable and healthy. The assessment of the tissues remains a difficult challenge for the pathologists due to the lack of early and non-specific changes to the tissues. We investigated using Quantum Cascade Laser (QCL) imaging to examine tissue biopsies from patients who have received heart and kidney transplants. In the kidney, we have identified a number of biochemical markers that are associated with the advancement of interstitial fibrosis, a component of chronic rejection, and that we can distinguish between the late and early cases for each group using the biochemical information. In addition, we have highlighted a 'biochemical- signature' that may be predictive of the later interstitial fibrosis, using baseline biopsies. In the heart allograft biopsies with immunopathologic antibody mediated rejection, IR spectroscopy reveals a biochemical signature unique to AMR compared to that of non-rejecting cardiac allografts and native hearts. Biochemical changes that proceed histological changes detected using IR spectroscopy may allow for early intervention to prevent organ loss.

**Abstract Text**

Transplantation remains the main treatment for end-stage organ failure. Due to the extreme demand for organs for transplantation it is critical that transplanted patients are closely monitored for complications to ensure that grafts remain viable and healthy. The assessment of the tissues remains a difficult challenge for the pathologists due to the lack of early and non-specific changes to the tissues. We investigated using Quantum Cascade Laser (QCL) imaging to examine tissue biopsies from patients who have received heart and kidney transplants. In the kidney, we have identified a number of biochemical markers that are associated with the advancement of interstitial fibrosis, a component of chronic rejection, and that we can distinguish between the late and early cases for each group using the biochemical information. In addition, we have highlighted a 'biochemical- signature' that may be predictive of the later interstitial fibrosis, using baseline biopsies. In the heart allograft biopsies with immunopathologic antibody mediated rejection, IR spectroscopy reveals a biochemical signature unique to AMR compared to that of non-rejecting cardiac allografts and native hearts. Biochemical changes that proceed histological changes detected using IR spectroscopy may allow for early intervention to prevent organ loss.

**Keywords:** Bioinformatics, Biomedical, Biospectroscopy, Spectroscopy

**Application Code:** Biomedical

**Methodology Code:** Biospectroscopy
Raman spectroscopy has been demonstrated across many clinical applications to be a powerful tool for the biochemical discrimination of disease. Translation of this technique to the clinic and enabling its use within the body is a difficult but achievable prospect.

Rapid, minimally invasive, in vivo molecular diagnosis would provide clinicians for a powerful tool. There are myriad clinical needs where such an approach would add significant value. These range from early diagnosis of malignancies on the linings of organs, to staging more advanced malignancies, to providing treatment monitoring, tumour margin, lymph node metastasis status and of course the prospect for prognostic signature identification.

Work over many years has taken the concept of in vivo Raman diagnostics to real clinical studies. A number of groups are now using the technique in vivo for the study of tissue composition and its association with diseased conditions. I will give an overview of some of our current work in the area.

This presentation will focus on a range of approaches:
1) Surface based analysis of disease specific tissue composition utilising endoscopic confocal Raman probes;
2) Subcutaneous analysis using needle Raman probes;
3) Deep Raman analysis of tissue composition with transmission and SORS approaches.

Abstract Text

Co-Author(s)
Monitoring cell secretion events remains a challenge to overcome in chemical analysis. Under stimulus, the molecules secreted by cells are related to the biological processes undergoing inside cells and thus monitoring the secretion of metabolites may provide important biological information. Cells may also secrete important chemical messengers to regulate interactions with neighbouring cells, which are also currently challenging to detect. Plasmonics provide an interesting alternative to electrochemical nanosensors for physiological measurements. The fabrication and properties of plasmonic nanopipettes with a diameter of 100 nm at the tip will be demonstrated for monitoring the extracellular media near cells. Au NP deposition using electrostatic interactions creates disordered arrays of agglomerated particles on the nanopipette, suited for SERS measurements. Molecules diffusing in the SERS hot spot experienced high electric fields and thus, high Raman response. The plasmonic nanopipette is thus competent for dynamic SERS measurements in the liquid environment near cells. The capability of precisely positioning the nanopipette near cells led to the dynamic measurement of the content of extracellular matrix in real-time. The detection of several metabolites, such as glucose, pyruvate, lactate and urea near cell will be demonstrated. Due to the high temporal resolution of the technique, real-time detection of cell secretion events was achieved for lactate and pyruvate secreted by live cells. This new nanosensor will provide physiological measurements of cell secretion events for electrochemically-inactive molecules.

Keywords: Bioanalytical, Biosensors, Raman Spectroscopy
Application Code: Bioanalytical
Methodology Code: Sensors
Our work has recently focused on the development of plasmonic platforms capable of detecting target biomarkers, with a goal towards rapid point-of-care (POC) diagnostics. Specifically, we have explored the modification of commercially available screen printed electrodes (SPEs) with carefully designed and synthesized metallic nanostructures, and have demonstrated a cost-effective and portable electrochemical surface-enhanced Raman spectroscopy (EC-SERS) set-up. Using this spectroelectrochemical system, we will present work on the rapid detection of a number of target biomarkers, including advanced aptasensor-based strategies for the direct detection of protein. In addition, work towards wearable and sustainable plasmonic healthcare technologies will be presented.
Chiral Method Development

Development and Optimization of a Method for HPLC-Separation of Enantiomers with Polysaccharide-Based Chiral Columns

This presentation summarizes our recent approaches for development and optimization of enantioseparation method in HPLC by using polysaccharide-based chiral selectors with especial emphases on enantiomer elution order and chiral analytes with multiple stereogenic centers. Aspects such as new kinds of chiral selectors, carrier materials, mobile phases, and mobile phase additives will be discussed. The effect of optimizing these parameters on separation efficiency, selectivity and analysis time will be demonstrated. Our newest results on the adjustment of the enantiomer elution order based on the chemistry of the chiral selector, the nature of the mobile phase, mobile phase additives and the separation temperature will be reported. In addition, an unusual increase of the separation selectivity based on the optimization of the structures of the analytes and the chiral selectors will be presented as well as baseline enantioseparations within a few seconds by using common HPLC instrumentation. In the final part, examples of enantioselective extraction with polysaccharide-based chiral materials will be shortly discussed.

Keywords: Chiral, Chiral Separations, HPLC, HPLC Columns
Application Code: Pharmaceutical
Methodology Code: Liquid Chromatography
The chromatographic behaviour of above 30 chiral stationary phases (CSPs) of varied nature (Pirkle-type, glycopeptide, polysaccharide and other polymeric phases) was assessed in supercritical fluid chromatography (SFC) with a large number of achiral (about 200) and chiral (about 100) solutes, based on a previously described method. First of all, the achiral solutes served to evaluate the interactions occurring between the stationary phase and solutes with the help of linear solvation energy relationships (LSERs) using a modified version of the solvation parameter model. The obtained models serve to compare the interactions contributing to retention on the different stationary phases. Secondly, the factors contributing to chiral separation on each CSP are assessed with factorial discriminant analysis. A rational tool is then available to unravel the factors affecting the quality of enantioseparation and compare stationary phases or operating conditions (composition of the mobile phase). For instance, it appears that some CSPs are more affected by solute shape than others, while resolution is generally impaired by increased flexibility of the solute. Also, the effects of halogen atoms of the chiral selector on hydrogen-bonding with the chiral analytes are evidenced.

A classification of CSPs used in SFC is then proposed. This should both help in the selection of the most appropriate chiral column when new chiral separations are required and allow for the definition of an orthogonal set of columns to avoid redundant experiments.

Keywords: Chemometrics, Chiral Separations, HPLC Columns, Supercritical Fluid Chromatography
Application Code: Pharmaceutical
Methodology Code: Supercritical Fluid Chromatography
Chiral Method Development

**Enantioseparation of Compounds with Multiple Chiral Centers by 2D-LC**

With the recent advances of chiral recognition stationary phases and chiral method screening strategy, chiral method development is significant more efficient and quicker than before. However, the complexity of molecules in pharmaceutical pipelines is increasing. The requests for enantioseparation of compounds with multiple chiral centers is increasing and getting more and more important in process control and drug impurity profiling. Typically, multiple methods are required to separate these chiral isomers, and even multiple methods not necessarily give the desired separation. The method development for these compounds is challenging, lengthy and tedious.

Two-dimensional HPLC is an effective way for resolving compounds with multiple chiral centers. We applied a platform strategy by separating the diastereomers in the first dimension using a high peak capacity achiral column, then heart-cutting the peaks of each pair of enantiomers and send to the 2nd dimension for further separation by chiral columns. This 2D-LC platform strategy significantly reduces the method development time and improves resolution. We have applied different 2D-LC separation modes such as RP-RP, RP-mRP, and RP-NP for different compounds with multiple chiral centers. Case studies will be presented for the support of in-process control and final API analysis.

**Keywords:** Chiral Separations, Chromatography, HPLC, Pharmaceutical

**Application Code:** Pharmaceutical

**Methodology Code:** Separation Sciences
The incorporation of chirality within biologically active molecules in pharmaceutical drug discovery has resulted in challenges using existing chiral stationary phases (CSPs). Several new phases, which were originally derived from polysaccharide chiral stationary phases and tested in the 1990’s, were recently created to explore the effect of fluorophilic retention mechanisms on the enantioseparation of halogenated compounds using SFC. Based on these results, new prototype phases have already been prepared on cellulose and evaluated by SFC using drug-like compounds. In this study, we will present the continuation of modifications made to the 3rd and 4th positions on the aromatic ring, with the preparation of 4-chloro-3-trifluoromethylphenyl carbamate and its comparison to the corresponding fluoro and trifluoromethyl counterparts. Furthermore, a new study to determine the effects of replacing methyl with trifluoromethyl, along with the comparison of 3,5-bis(trifluoromethylphenyl) carbamate to the corresponding dimethylphenyl carbamate of cellulose (ChromegaChiral CCO) will be presented. We will also explore the use of pyridine as a replacement of the phenyl carbamate to determine if it is suitable for use in chiral (or achiral) separations.

Keywords: Chiral Separations, Chromatography, Separation Sciences, Supercritical Fluid Chromatography
Application Code: Pharmaceutical
Methodology Code: Supercritical Fluid Chromatography
Chiral Method Development

**Chiral HILIC? Unique Enantioselectivity Between HILIC and RP Mode Separations with Polysaccharide-Based Chiral Selectors**

Polysaccharide-based chiral selectors (CS) are currently used extensively in a variety of separation modes such as NP, SFC, PO, RP and PI. Each of these modes use the same CSs but in mobile phases (MP) of markedly different composition and based on apparently different separation mechanisms. Also, CSs demonstrate very different rates of success in various separation modes.

Whenever aqueous mobile-phases are used in combination with polysaccharide-based CSs, it is a expected that the separation is based on reversed-phase selector-selectand interactions. In contrast, our recent studies on various groups of chiral compounds indicate that with a water content of up to 20% in the mobile phase most polysaccharide-based CSs operate based on HILIC-like - rather than RP - separation mechanism. This can be amply demonstrated with chiral compounds of pharmaceutical importance such as dihydropyridine derivatives, imidazole and triazole-based antimycotic agents, -blockers, arypropionic acid derivatives, coumarines, barbiturates, etc. Enantiomer retention decreases with increasing water content in the mobile phase and this effect is most apparent in acetonitrile-water rather than in methanol-water (in line with typical HILIC mode separations). This behavior underlines the importance of hydrogen bonding in chiral recognition with polysaccharide-based CSs. At a water content higher than 20%, hydrophobic interactions seem to govern the selector-selectand interactions rendering such enantioseparations of the RP type.

Furthermore, for several chiral compounds reversal in enantiomer elution order was observed with increasing the content of water in the mobile phase (coumatetralyl, warfarin and hexobarbital on Lux Cellulose-1, pirprofen on Lux Cellulose-2, ibuprofen and warfarin on Lux Cellulose-3, ketorolac on Lux Amylose-1, etc.) indicating a dramatic change in chiral recognition mechanisms.

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

We employ an overall chiral separation strategy divided into three parts: (1) screening through the use of multiple HPLC chromatographic modes and various chiral columns in the context of an automated screening system, (2) thorough optimization of promising separations to achieve HPLC conditions for method validation and (3) continual investigation of new technologies for improving the current chiral method development process. The screening system will be outlined followed by examples of the optimization approach. Finally, the research into pressurized planar electrochromatography (PPEC) as a chiral screening technique will be described.

### Keywords
- Chiral
- Chromatography
- HPLC
- Pharmaceutical

### Application Code
- Pharmaceutical

### Methodology Code
- Separation Sciences

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**Session Title**: Chiral Method Development

**Abstract Title**: Chiral Separation Screening, Optimization and New Technology – An Ongoing Chiral Method Development Process

**Primary Author**: Donald S. Risley

**Eli Lilly and Company**

**Co-Author(s)**: Megan A. Gokey, V Scott Sharp

**Date**: Thursday, March 09, 2017 - Afternoon

**Time**: 03:25 PM

**Room**: W183c
Chiral Method Development

Using Blends of Solvents and Additives to Enhance SFC Chiral Method Development Screening

SFC chiral method development usually involves screening numerous chiral stationary phases (CSPs) in search of one providing adequate selectivity between stereoisomers. To keep CSP screening simple, many analysts employ single solvents instead of mixtures and also dispense with additives. However, this approach forgoes the benefits of selectivity/resolution modulation from judicious blending of solvents and additives. Blending two or more solvents, with or without additives, has been occasionally described in SFC but little investigated for improving selectivity/resolution between enantiomers on chiral stationary phases.

In this presentation, we discuss a systematic study with forty four different blends of solvents and additives. These blends used selected combinations of common SFC solvents (methanol, ethanol, isopropanol and acetonitrile) plus one of three pH modifying additives (trifluoroacetic acid, ammonium acetate and ammonium hydroxide). Fifty five diverse racemate probe compounds were analyzed on three sub 3 μm particle polysaccaride CSPs with these blends to determine which may enhance the enantiomer separation. This resulted in a four injection screen with blends that outperformed individual solvents for chiral resolution.

Abstract Text

Keywords: Chiral, Method Development, SFC, Supercritical Fluid Chromatography
Application Code: Other
Methodology Code: Supercritical Fluid Chromatography
Chiral HPLC method development must be approached differently than achiral HPLC method development. Although complex, achiral analyte - stationary phase interactions can be predictably identified and utilized for the development of chromatographic separations. However, the interactions between chiral analytes and chiral stationary phases are not identifiable or predictable. Experimental testing is the only means of determining chiral selectivity for an analyte with a particular chiral stationary phase. Chiral HPLC method development always begins with screening different chiral stationary phases.

Preparative HPLC is similar to analytical HPLC in many aspects but there are significant differences. The main objective for Prep HPLC is to isolate material while the main objective for analytical HPLC is the resolution of particular components. The Prep HPLC method development goal is to optimize methodology based on desired purity, yield, and throughput. The Analytical HPLC method development goal is to optimize resolution with suitable detection and run time. Solubility of the initial and isolated material is a significant issue in Prep HPLC and must be address during method development. Solubility is rarely an issue with Analytical HPLC. Isolation of the final material must be another consideration during Preparative HPLC method development. The stability of the desired material needs to be maintained during the entire Prep HPLC process.

The work presented here will use several real world Pharmaceutical examples to demonstrate strategies for developing chiral preparative chromatographic methodologies. These examples will highlight column screening, method optimization, sample introduction techniques, material isolation techniques and yield estimation during the development.

Keywords: Chiral Separations, Method Development, Pharmaceutical, Prep Chromatography
Application Code: Pharmaceutical
Methodology Code: Liquid Chromatography
Carbon electrodes are routinely used for electrochemical detection and application in electrochemical sensors to quantify electroactive analytes in a variety of media. By electroactive, one is referring to molecules that are easily oxidized or reduced at an electrode surface. Generally speaking, electrochemical measurements often involve application of a potential to an electrode and measuring the current that flows in response to a potential perturbation, which is reflective of the local analyte concentration. Carbon is one of the most abundant elements found on the planet and, from a materials perspective, is unique because of the microstructurally-distinct allotropes it forms. These range from single and polycrystalline diamond, to the stacked sheets of graphite, to the microstructurally-disordered glassy carbon, to nanotubes and fullerenes, and finally to the single sheet graphene. All of these carbon materials are used in electrochemical measurements as well as other technologies, in part, because of some common attributes: high mechanical strength, good thermal conductivity and stability, chemical inertness, high carrier mobility and good electrical conductivity, and rich surface chemistry.

Boron-doped diamond (BDD) is one type of carbon electrode that performs well in electroanalytical measurements, often providing superior detection figures of merit compared with conventional carbon electrodes like glassy carbon. In addition, BDD can function as an optically transparent electrode for transmission spectroelectrochemical measurements. In this presentation, some of the basic material and electrochemical properties of this unique electrode material will be reviewed and some examples of how BDD has been used (i) for the determination of trace metal ions in solution by anodic stripping voltammetry and (ii) as an optically transparent electrode for transmission spectroelectrochemical measurements will be highlighted.

**Keywords:** Analysis, Bioanalytical, Electrochemistry, Spectroelectrochemistry

**Application Code:** Bioanalytical

**Methodology Code:** Electrochemistry
Electrically conductive low-dimensional nanomaterials hold promise for enabling the fabrication of portable chemical gas sensors with the goal of improving human health, safety, and quality of life. Although gas sensors based on carbon nanotubes and graphene have found several applications in detecting the presence of gases and vapors, several challenges currently inhibit rapid progress towards technological applications. These challenges include limited access to structurally well-defined materials and requirement for post-synthetic modification in the solids state. An emergent class of two-dimensional materials based on electrically conductive hybrid organic-inorganic constructs--metal-organic frameworks (MOFs)--present an alternative strategy for accessing low-dimensional stimuli-responsive nanomaterials with utility in electronic chemical sensing. This presentation will describe several approaches for integrating metal organic frameworks into portable device architectures, and utility of these devices in gas detection and capture.
Nanodiamond (ND) particles have recently emerged as a key platform for many sectors of nanoscience and nanotechnology due to their outstanding mechanical performance, biocompatibility and distinctive optical properties, a combination of assets not often met in the nanoworld. Optically active NDs remain one of the most popular research topics mainly due to the photoluminescent properties of crystallographic defects in the diamond lattice, referred to as color centers. Recently our group succeeded in large scale production of fluorescent NDs containing nitrogen-vacancy (NV) color centers in a hundred of grams batches. Production of fractions of ND-NV with median sizes ranging between 10 and 100 nm was achieved. Relative brightness of nanoparticles of different sizes and surface termination will be summarized. Functionalization of small size ND-NV with biomolecules preventing their aggregation will be demonstrated and stability in cell culture media discussed. Small size biofunctionalized nanodiamonds have perspectives as highly photostable and non-toxic fluorescent labels and sensors in life science applications.

Acknowledgment: NIH SBIR Phase I and Phase II Contract HHSN268201500010C.

Keywords: Bioanalytical, Biosensors, Fluorescence, Materials Characterization
Application Code: Bioanalytical
Methodology Code: Fluorescence/Luminescence
Amorphous carbon (aC) films can be deposited at room temperature and are chemically stable under ambient conditions or when interfaced with aqueous solutions, making them a cost-effective material for developing chemically modified electrodes. A number of wet chemical methods have been utilized to tailor the reactivity and wettability of aC films, but few of these chemistries are compatible with photo-patterning. Here, we introduce a method to install thiol groups directly onto the surface of aC films. These thiol groups are compatible with thiol-ene click reactions, which allowed us to rapidly pattern the surface of the aC films and prepare modified electrodes. We thoroughly characterized the aC films throughout the two-step thiolation reaction and confirm the installation of surface bound thiols without significant oxidation or changes in surface topography. We determined the best conditions for selectively attaching alkene-containing groups to aC films: the presence of thiol groups on the surface and alkene-group in the molecule of interest as well as illumination with near UV light. We also found this surface-bound thiol-ene reaction does not require the addition of a radical initiator. To demonstrate the utility of our approach, we confirmed our ability to photo-pattern the aC films with scanning electron microscopy and prepared ferrocene-modified aC electrodes. Thiol-terminated aC films will allow for the rapid attachment of different functional groups to the surface, allowing for the fabrication of sensors and patterned arrays of (bio)molecules on stable carbon interfaces.
Carbon materials are highly versatile and find numerous applications in energy conversion and storage. Effective control over interfacial properties via bulk or surface modifications is often critical to achieve their desired performance; in particular, modification via nitrogen incorporation is interesting for energy applications as nitrogenation has been reported to increase electrocatalytic performance in the oxygen reduction reaction (ORR). ORR is a redox process that is critical for several fuel-cell and battery technologies and nitrogenation has been shown to both improve the catalytic activity of carbon-supported metal nanoparticles and to impart intrinsic electrocatalytic activity. However, the origin of this activity is not well understood and rational optimization of carbon chemistry and structure for ORR remains elusive due to the interplay among electronic properties, surface chemistry and morphology changes associated to the introduction of N-sites.

This presentation will discuss the use of synthetic strategies in our group for improving our understanding of the role of N-sites in the electrocatalysis of the ORR at carbon electrodes. First, chemical vapour deposition was used to create graphene-like nanostructured carbon with tailored edge and defect density. A combination of microscopy, spectroscopy and electrochemical methods was used to correlate carbon chemistry and morphology to ORR activity in alkaline media. Results suggest a strong positive correlation between activity and density of electroactive edge sites; also, electrochemical methods were found to more reliably predict ORR activity than microscopy or spectroscopic methods. Second, a combination of sputter deposition and thermally-driven reactions was used to fabricate carbon electrodes with a single type of N-site and with varying degrees of disorder in the carbon scaffold. These electrodes were used as model carbon materials to test the role of N-site chemistry in ORR.

Keywords: Electrochemistry, Electrode Surfaces, Electrodes, Energy
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Electrochemistry
During the past few years we have prepared and reported microfabricated thin layer chromatography (TLC) plates based on carbon nanotube (CNT) templates. These plates were produced by patterning thin films of alumina and iron onto substrates, annealing the iron in a reducing environment to produce iron nanoparticles, growing the CNTs with a hydrocarbon precursor from the iron nanoparticles, infiltrating the resulting patterned CNT forests with an inorganic material (silica or a silica precursor), burning out the CNTs, and rehydroxylating the silica [1-5]. Various gas phase deposition techniques were explored for depositing silica on the CNT scaffolds. The plates produced in this manner showed high efficiencies and speeds. While significant and continual improvements were made in the preparation of these plates, the series of steps necessary to prepare them, along with the specialized equipment that would be required, makes manufacturing a fairly daunting task. Accordingly, we have been exploring new methods that will yield similar high quality plates while being much more manufacturable. This talk will describe our latest efforts along these lines.


Keywords: Chromatography
Application Code: Drug Discovery
Methodology Code: Liquid Chromatography
The interaction of nanomaterials with environmental and/or biological systems is complicated by the fact that most nanomaterials transform in the environment, either through changes in the chemical composition of the nanoparticle core or via acquisition of various coatings, often referred to as "coronas". Recently, the use of nano-diamond has attracted attention because diamond surface chemistry is extraordinarily robust, and because the intentional introduction of substitutional nitrogen and vacancies leads to the formation of Nv centers. Nv centers are fluorescent and allow nano diamond to be used as an unbleachable fluorescent probe. In this talk, I will discuss the use of Nv-labeled nano diamond for investigations of nanoparticle behavior in environmental and biological systems, including the use of optically detected magnetic resonance to enhance the ability to selectively detect diamond nanoparticles in complex matrices.

Keywords: Environmental Analysis, Environmental/Biological Samples, Imaging, Luminescence
Application Code: Environmental
Methodology Code: Fluorescence/Luminescence
Modified Carbon-Based Materials for Sensors, Arrays, and Catalysis

Impact of Thickness and Short-Range Interactions on the Electrochemical Response of Ultra-Thin Graphene Interfaces

Bulk and surface electrochemical reactivity converge on ultrathin electrochemical interfaces such as monolayer and few-layer graphene (FLG). We will demonstrate that graphene enables unique chemical interactions that operate at scales coincident with its thickness. These electronic, electrostatic, and chemical interactions result in modified ion intercalation, and enhanced rates of electron transfer for outer- and inner-sphere electrocatalytic processes, as shown in Figure 1.

In a first study, we used scanning electrochemical microscopy (SECM) to demonstrate unambiguously that SLG electrodes deposited over micro-patterned metallic substrates exhibit facilitated electron transfer to mediators in solution.[1] In a second study, we demonstrate that thickness effects are not constrained to electron transfer. We used FLG of low defect density and thickness of 2-8 layers to explore its alkaline ion intercalation mechanisms.[2] Cyclic voltammetry and novel ion-sensitive SECM measurements suggest a different staging mechanisms of alkaline ions intercalating into graphene with different layer number. These studies highlight the unique electrochemistry attainable with graphene. Thus, beyond its superior electronic properties, graphene opens new frontiers in the design of materials for catalysis, surface patterning, and sensing.


Non-mammals, such as fruit flies and sea anemones, offer new model systems and thus new challenges to further understand how molecular changes associated with neurotransmission work to influence behavior. Signaling molecules such as hormones, neurotransmitters metabolites, and precursors exist in complex media at low concentrations which renders them difficult to analyze, so separations with either a highly selective detector such as coulometry or significant sample preparation such as solid phase extraction must be used. Two mobile phases were optimized for their use in the quantification of 20 neurotransmitters: 1) a phosphate-based “MDTM” buffer which is a 75 mM phosphate/4% acetonitrile solution at pH 3.00 and 2) a citrate-acetate buffer that contains 100 mM sodium citrate, 90 mM sodium acetate, 0.1% acetonitrile, at pH 9.00. The two mobile phases were then used to measure neurotransmitter levels in dissected brains of aged-matched fruit flies. For the detection of phytoestrogens such as isoflavones, which have been thought to affect reproduction of sea life, solid phase extraction was developed for use with a formic acid- acetonitrile gradient for high performance liquid chromatography with mass spectrometry separation. Three isoflavones: diadzein, genistein, and biochanin A were investigated in sea anemones containing symbiotic and aposymbiotic algae. It is hypothesized that the symbiotic relationship between these anemones and their algal counterparts may regulate the method sea anemones reproduce, specifically the algae release compounds that decide whether the anemones reproduce sexually or asexually. Samples tested included both supernatant and pellet specimens of sea anemone with and without algae, supernatant and pellet samples of the algae itself, and water samples from anemone cultures. It is hoped that the methods described here will provide greater insight into how behavior is affect by the development of signaling pathways in these species.

Keywords: Bioanalytical, Chromatography, Electrochemistry, Mass Spectrometry
Application Code: Bioanalytical
Methodology Code: Liquid Chromatography
Monoclonal antibodies, such as Trastuzumab, are used for treatment of breast cancer. However, the glycosylation of monoclonal antibodies varies with manufacturing protocol (1). Glycosylated antibodies may contain sialic acid, fucose or galactose residues. Analyzing the glycosylation can be important in therapeutics. Trastuzumab targets the HER2 receptor overexpressed by SKBR-3 breast cancer cells (2). The goal of the current study is to determine how glycosylation of trastuzumab affects its binding to SKBR3 cells and its potency. Lectin affinity chromatography is used to fractionate the antibodies depending on glycosylation. Nanomolar range of antibody is used hence the trastuzumab will be labeled and detected with fluorescence. The binding effectiveness of the fractionated antibodies to SKBR3 cells is assessed with different concentrations of the target cells using a flow cytometer to determine the Kd. The potency of the different antibody fractions is determined by antibody dependent cell-mediated cytotoxicity and complement dependent cytotoxicity. Antibody dependent cell-mediated cytotoxicity is determined by incubating fluorescent labelled SKBR3 cells with antibodies and labeled effector cells at different effector cell concentrations. Natural killer and B cells are used as effector cells because they target and lyse SKBR3 cells bound to antibodies. A flow cytometer is used to identify and quantify dead/live SKBR3 cells and effector cells depending on the labeling they have. Complement dependent cytotoxicity is assessed by culturing antibodies and labelled SKBR3 with human complement for different time periods. After incubation dead and live SKBR3 cells are quantified in a flow cytometer depending on different labeling.

References
2. Miguel A. Molina, Jordi Codony-Servat, Joan Albanell, Federico Rojo, Joaquín Arribas, and Jose Baselga, Cancer Research, 2001, 61, 4744–4749,
Quantifying the binding of biologically relevant analytes to receptors is important for understanding cellular signaling pathways. However, direct binding of many signaling molecules to receptors is difficult to monitor without the use of separation techniques or labels. Radiolabels offer advantages over traditional labels (i.e. fluorophore or electrochemical labels), particularly tritium (3H), because of minimal perturbation to the structure, mass, and binding characteristics of parent molecules. 3H-labeled compounds may be detected through scintillation, a process that converts low-energy particles emitted during radioactive decay into detectable photons. Traditionally, solid scintillation counting using scintillant-doped multiwell plates with immobilized receptors has been used to quantify binding of radiolabeled analytes. Alternatively, we developed multifunctional nanoparticles for scintillation proximity assay (nanoSPA). Nanoparticles offer higher surface area for binding, which can lead to enhanced scintillation efficiency. Moreover, nanoparticles are amenable to various surface coatings, such as lipid bilayers to allow membrane receptor insertion for binding studies and to reduce nonspecific adsorption. Receptors immobilized onto the nanoSPA capture radiolabeled analytes in close proximity to the nanoparticle and facilitate separation-free detection of radiolabeled analytes. Here, we describe the fabrication of a nanoSPA assembly that uses a polystyrene-core silica-shell structure, coated with a phospholipid bilayer for receptor insertion to study receptor-ligand pairs. Using this assembly, different ligand-receptor pairs (e.g. NeutrAvidin-biotin) were explored to quantify binding. Six-fold enhancement was observed for target analytes vs. non-proximity effects, and the coating minimized nonspecific adsorption by 89% vs. bare particles. The nanoSPA assembly may be a versatile platform for high-throughput screening of receptor-ligand pairs.
Bioanalytical - LC, Sensors, and Microscopy

**Abstract Title**
Silicon Photonic Microring Resonators for the Multiplex Detection and Quantification of Non-Coding RNA

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**Abstract Text**
The aim of the research is the use of a Silicon Microring Resonator Platform for the detection and quantification of diverse RNAs with medical relevance, such as micro-RNAs (miRNAs) and long non-coding RNAs (lncRNAs). For precise quantification of RNA, Reverse Transcription and Polymerase Chain Reaction (RT-PCR) are performed starting from isolated RNA. The biosensor chip consists of an array of 32 clusters of microrings that can be individually functionalized allowing a multiplex detection of RNAs. By coupling PCR to the biosensor platform, we can achieve the detection of very low amounts of nucleic acids with increased signal at higher number of PCR cycles. The sensors response as a function of PCR cycle can be directly related to the initial concentration of RNAs; therefore, this methodology is comparable to RT-qPCR but in a label-free manner. The biosensor platform was used in the detection of different miRNA targets with relevance in glioblastoma, a highly malignant brain cancer. We measured the expression of cancer-related miRNA in healthy patients and compared to patients with different grades of glioma. In addition to miRNA, we extended our work to the detection of lncRNAs in the same RNA samples, in order to find more features for the classification of patients. In conclusion, the results demonstrate the capability of the platform for the detection of RNA and the possibility of measuring very low concentrations of multiple targets in a label-free way.

**Keywords:**
Bioanalytical, Biosensors, Detection, Quantitative

**Application Code:**
Bioanalytical

**Methodology Code:**
Sensors
Characterization of Liposomal Loading for Biosensor Development Using Tethered Small Unilamellar Vesicles

Phospholipid vesicles (liposomes) have been extensively used in intracellular delivery, bioreactor, and biosensor applications. Development of biosensors requires uniform integration of sensing components, including proteins, enzymes, as well as non-destructive approaches to stabilize the sensor architecture that are compatible with sensor function. We used total internal reflection fluorescence (TIRF) microscopy of polymer stabilized small unilamellar vesicles (SUVs) to investigate the effect of vesicle stabilization and the loading uniformity of fluorescent protein in model biosensor architectures. Characterization of SUVs containing biomolecules represents a significant challenge due to the sub-diffraction size and the fusion probability on solid (glass) surfaces. The model biosensor platform was prepared via incorporation of green fluorescent protein (eGFP) and red fluorescent protein (td-Tomato) in stabilized synthetic SUVs containing ~1 mol% of biotin labelled phospholipid. Protein loaded SUVs were immobilized on planar supported lipid bilayer (PSLB) formed at the plasma cleaned hydrophilic glass surface via biotin-avidin chemistry. SUVs were stabilized via partition and subsequent polymerization of hydrophobic methacrylate monomers in presence of photoinitiator, redoxinitiator and thermoinitiator individually, and their effects on function and stability of eGFP and td-Tomato were evaluated. Once the stabilization conditions were optimized, SUVs loaded with fluorescent proteins under different solution conditions were analyzed to quantify the individual SUV compositions. These results will be used to drive future biosensor design by optimizing the composition of individual nanometer-sized sensor architectures.

Keywords: Bioanalytical, Biosensors, Membrane, Spectroscopy
Application Code: Bioanalytical
Methodology Code: Sensors
### Abstract Title

**Multiplexed Microring Resonator Arrays to Characterize Biological-Based Therapeutic Agents**

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**Abstract Text**

There has been a significant increase in the development of biological-based therapeutic agents. The most pervasive class of biological therapeutics is humanized monoclonal antibodies (mAb), which can treat a variety of disorders via selective inhibition or stimulation of biological targets. Antibody-based therapeutics present an analytical challenge to validate the purity and efficacy compared to traditional small molecules. The chemical structure is insufficient characterization, but rather properties that effect binding, affinity, and specificity must be determined. The critical parameters defining the efficacy of a mAb therapy include degree of aggregation and epitope specificity. Currently available analytical methods are cumbersome and difficult to integrate into the development pipeline.

We have developed a workflow coupling size exclusion chromatography (SEC) to our multiplexed microring resonator detection platform to evaluate the functional binding characteristics of therapeutic monoclonal antibodies. Sensor arrays feature a combination of capture probes targeting the IgG constant regions as well as Fc and Fab fragments. By fluidically coupling SEC to the microring resonator platform, we can fractionate discrete aggregate populations and assess the affinity reagent-specific responses in a single workflow. We have evaluated the binding characteristics of a dimeric therapeutic mAb population to elucidate the dimer structures and by extension the effect of dimerization on target recognition. This combination method will be a powerful addition to the product development and quality assurance pipelines that support the emerging field of biological therapeutics.

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**Keywords:** Bioanalytical, Chromatography, Method Development, Sensors

**Application Code:** Bioanalytical

**Methodology Code:** Sensors
Development of a pH Sensor for Non-Invasive In Vivo Detection and Imaging of Implant Associated Infection

We develop a pH sensor based on the use of X-ray Excited Luminescence Chemical Imaging (XELCI) to noninvasively diagnose and monitor implant-associated infection in situ. Early diagnosis of implant-associated infection and noninvasive continuous monitoring of infection is a challenge and the treatment is highly dependent on the detection of infection at its onset. Bacteria and inflammatory responses cause a pH drop in affected area and pH shifts to acidic from physiological pH which can indicate infection. Our pH sensor uses a combination of X-ray excited optical luminescence and pH-dependent optical absorption to detect pH changes through tissue. It consists of a layered structure of a pH sensitive polymer film over scintillator particles (Gd2O2S:Eu) which act as an in situ X-ray irradiated light source emitting red light which is differentially absorbed by the pH sensitive film on top depending on pH of the area. The pH sensor is characterized for reversibility, sensitivity and resolution. We studied pH changes during formation of biofilm on the pH sensitive sensor film in vitro. We also tested the pH sensor in animal model and cadaver studies. XELCI allows to noninvasively detect and image changes in pH at implant surface with high spatial and pH resolution while minimizing tissue scattering effects. It allows point by point mapping of the surface with minimum background and high spatial resolution mainly limited by X-ray beam width. In summary, our sensor provides a novel approach to non-invasively image surface pH to diagnose implant infection and assess treatment.

Acknowledgements: This research was supported in part by National Science Foundation (NSF) CAREER award CHE1255535, a Fulbright Scholarship award to Unaiza Uzair, and animal studies funded through the South Carolina Bioengineering Center of Regeneration and Formation of Tissues (SCBioCRAFT) funded under NIH grant R15EB014560-01A1.

Keywords: Biosensors, Imaging, Luminescence, Surface Analysis
Application Code: Bioanalytical
Methodology Code: Sensors
Ischemic stroke has been found to cause different levels of cell death in hippocampal Cornu Ammonis (CA) subfields such as CA1 and CA3 in different mammalian animal models including human, but the mechanism behind this has not been determined. We hypothesize that reactive oxygen species (ROS) could be involved since abundant evidence shows that escalation of ROS by ischemic stroke results in cell death. Under this inspiration, we wish to study the early stage of ischemic stroke within 1h in CA1/CA3 to determine whether they are different.

Organotypic hippocampal slice cultures (OHSCs) and oxygen-glucose deprivation (OGD) and reperfusion (RP) are used as our tissue and stroke model, respectively. Changes of [H2O2] (a type of ROS) and glutathione (GSH, as an antioxidant) are measured using GFP-based sensors in mitochondria and cytosol of pyramidal cells in OHSC. A combination of menadione and superoxide oxide dismutase mimic have been applied to stress OHSCs in a similar way as OGD-RP. Auranofin is used as an efficient inhibitor of thioredoxin reductase. We find that CA1 mitochondria are more oxidizing than CA3 ones. These differences are abolished following with auranofin. Meanwhile, the content of Trx (especially its mitochondrial subtype Trx2) is significantly higher in CA3 than CA1 while such a difference is not observed in other related proteins. Our results show that Trx contributes to the CA1/CA3 difference in response to oxidative stress.

Supported by NIH Grant R01 GM066018.

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Supported by NIH Grant R01 GM066018.

Keywords: Bioanalytical, Imaging, Immunoassay, Microscopy
Application Code: Bioanalytical
Methodology Code: Microscopy
Sepsis, systemic inflammatory response syndrome (SIRS), and septic shock are some of the most frequent causes of death in hospitalized patients. In confirming cases of suspected sepsis, blood culture is considered as the “gold standard”. However, blood culture has drawbacks such as time-consuming (2 to 4 days), prone to error from false-negative outcomes from previously received antibiotics, and has the possibility of pathogens not growing in available culture media. FcRI (CD64) has shown to be a sensitive and specific biomarker for diagnosis of bacterial infection and is a promising sepsis marker. In this work, an affinity capture method was developed to capture cells based on CD64 expression differences in a single microfluidic chip. HL-60 cells were differentiated to neutrophils (dHL-60) with DMSO. IFN-γ was used to upregulate CD64 expression on dHL-60 cells to simulate septic conditions and increased CD64 expression. The mixture of dHL-60 cells and IFN-γ-regulated dHL-60 cells was captured using herringbone-modified capture channels at stop flow. The separation purity reached 96% and the IFN-γ-dHL-60 cells enrichment was 5×. We observed that the capture of dHL-60 cells and IFN-γ-dHL-60 cells was consistent with CD64 expression on each cell line. This device was capable of monitoring sepsis development along the time and was promising to detect sepsis earlier than extant methods.

Keywords: Bioanalytical, Lab-on-a-Chip/Microfluidics, Method Development
Application Code: Bioanalytical
Methodology Code: Chemical Methods
Chemical Methods (Half Session)

Selenophene with a Basic Side Chain: A New Core Structure for Subtype-Selective Estrogen Receptor Ligands

Estrogen receptor (ER) is regarded as an important pharmaceutical target for the treatment of breast cancer, and development of ER ligands has emerged as an active study field in the fight against breast cancer. Many of these ligands often have mixed agonist-antagonist and tissue-selective activities, some of which have been termed selective estrogen receptor modulators (SERMs).1

In the development of both SERMs and subtype-selective ligands, extensive investigation has been conducted to non-steroidal compounds having heterocyclic cores. As part of our ongoing interest in the development of ER ligands with different core structures, recently we have developed a series of novel ER ligands based on a thiophene core, and some of the diarylthiophenes show distinct superagonist activity in reporter gene assays, giving maximal activities 2-3 times stronger than that of estradiol.2 More recently, we found for the first time that the selenium-containing heterocycle: selenophene, a bioisostere of thiophene could also be used as a core structure for estrogen receptor ligands. In order to enhance the ER binding affinity, subtype selectivity, and antiproliferative activity of these selenophenes, we reported herein a series of novel selenophene derivatives containing a basic side chain as estrogen receptor ligands (Scheme 1). As we expected, these compounds show good subtype selectivity for ER binding affinity, and in transcription assays, the results suggest that these derivatives largely exhibit partial or full ER antagonist activity. Very interestingly, compared with the approved anti-breast cancer drug 4-hydroxytamoxifen, several compounds exhibited superior antiproliferative potency in breast cancer MCF-7 cell lines.

References

Keywords: Drug Discovery
Application Code: Drug Discovery
Methodology Code: Chemical Methods
Exposure to toxic volatile organic compounds (VOCs) has been shown to have negative health consequences making the analysis of VOCs in whole human blood an important tool for exposure assessment. Even though a number of whole blood components are tightly regulated by the body, certain behaviors and physiologies can cause variation among individuals. Theoretically, lipid concentration has the potential to affect the solubility of VOCs in blood, especially for nonpolar compounds such as the alkanes and dichlorobenzenes. Previous publications have discussed the need for a lipid correction when quantifying VOCs in whole blood, leading us to explore whether such a correction would have a significant effect on quantification accuracy for an analytical method that uses isotopically labeled internal standards. The aforementioned lipophilic compounds were compared to other, more polar VOCs to examine the influence of blood lipid on VOC quantification. Preliminary results from National Health and Nutrition Examination Survey (NHANES) data for blood VOCs and blood lipid levels, as well as laboratory experimental values, show that blood lipid concentrations do not have a significant impact on VOC levels. Therefore, based on these initial results, a lipid correction is unnecessary when measuring and reporting VOC levels in blood. Both the NHANES and experimental data were collected using headspace sampling by SMPE followed by GC/MS analysis.

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

Application Code: Bioanalytical

Methodology Code: Data Analysis and Manipulation
Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disorder, resulting in muscle atrophy, loss of motor function, and eventually death. As the disease currently has no known biomarker, assessment of disease progression is performed using different types of rating scale and questionnaires. This method of assessment is coarse and can be subject to bias, thus hampering the development of drugs and therapeutics for treatment.

As the motor neurons become diseased they struggle to hold their membrane potential, causing spontaneous discharges known as fasciculations. The electrical waveform, the Fasciculation Potential (FP), can be captured using electromyography (EMG). Evidence has been found to show that variability in the FP waveform [1], the frequency, and occurrence of fasciculations can be linked to the progression of the disease. Development of a quantitative measure of ALS progression using FP analytics may be of use as a surrogate biomarker to determine disease progression and therapeutic response to prospective drugs and therapies.

EMG recording sessions can be very long in duration, capturing gigabytes of data. As these recordings will potentially contain many FPs, manually sorting through the data is not a feasible solution. This is a general problem with large datasets of high frequency analytical data. Our solution is to use a combination of novel algorithms and established machine learning techniques in order to automatically process EMG recordings and group FPs by their motor unit. Once correctly grouped, the relevant information from each fasciculating motor unit is extracted for analysis. A multiple channel, wireless device is in development in order to capture both temporal and spatial EMG data using high-density surface EMG grid arrays. The aim is to develop the device into a 'smart sleeve' that can be worn by the patient for long periods of time.

1. De Carvalho M, Swash M. J Neurol Neurosurg Psychiatry 2013; 84(9):963–968

Keywords: Bioanalytical, Biomedical, Data Analysis, Data Mining
Application Code: Bioanalytical
Methodology Code: Data Analysis and Manipulation
Before analyzing an accurate-mass MS/MS spectrum of an unknown compound, it is good practice to find fragment ion masses that may not actually belong in the spectrum.

There are five sources of extraneous ion masses: bad calibration, electronic noise, chemical interference from background compounds in the mobile phase, chemical interference from co-eluting compounds, and unexpected reactions in the collision cell.

The first two sources (“extraterrestrial ions”) can be detected graphically with a “rule of thumb”. Extraterrestrial ions have masses that do not make any sense for the elements C, H, N, O, S, P, F, Cl, and Br. If one plots the maximum positive ion defect versus the nominal mass and the minimum negative ion defect versus the nominal mass on the same graph, the cone of extraterrestrial mass defects is obtained. No fragment ion or neutral loss should be found with a mass defect that is inside the cone. Based on the masses of the elements C, H, N, O, S, P, F, Cl, and Br, the masses between 4 and 14 and between HF (20.0062) and cyanide (26.0031) can be excluded.

Chemical interferences usually creep into a spectrum from the mobile phase. Certain fragment ion masses are strong indications of mobile phase interferences.

Artifacts can also arise if nitrogen is used as the collision gas. Although nitrogen is usually regarded as an inert gas, it will react with arylium ions. This rather serious problem can be avoided completely by using argon as the collision gas.
In previous work, Raman spectroscopy together with statistical modeling was shown to be effective for real-time data acquisition of consumable sugar (glucose) and accumulating products (butyric acid, acetic acid, and butanol) in a clostridial fermentation culture. Developed partial-least squares (PLS) models were applied to both agitated and static cultures with the former showing preferred modeling parameter values ($R^2_Y = 0.99$ and $Q^2_Y = 0.98$). Model outputs were shown to be comparable to off-line analyzed data from traditional HPLC for new clostridial experimental data through cross-validation. In this study, a bottom-up approach is employed where experimental data from HPLC analyzed data for reaction components is used to simulate an artificial fermentation culture devoid of cell activity. Raman spectra of corresponding reaction components; (i) glucose, (ii) butyric acid, (iii) acetic acid, and (iv) butanol, in specified proportions were acquired for corresponding time points. The acquired spectra together with known concentrations of reaction components were used to build new sets of PLS models. These new models will be compared with original models created for the actual clostridial fermentation for model performance with both sets of models executed in real-time.

**Keywords:** Bioanalytical, Chemometrics, Process Monitoring, Raman Spectroscopy

**Application Code:** Bioanalytical

**Methodology Code:** Computers, Modeling and Simulation
We had a need to computerize several different kinds of devices in our laboratories. We make many different kinds of measurements, and need flexibility to meet the demands of various projects. Instrument companies offered specialized, inflexible, and expensive software for narrow applications; hardware companies, on the other hand, offered good hardware but clumsy, inflexible, and expensive programming environments. As no complete commercial offerings would meet our needs at a reasonable cost, we chose to develop an easy-to-use, flexible user interface for all our measurements. We wanted a complete instrument interface system which would (1) take in several types of signals, (2) provide real time graphics for the user, (3) provide real time instrument control, (4) do data post-processing and archiving, (5) not require specialized programming, and (6) be obtainable at modest cost. We have developed such a system. The instrument interface system consists of a combination of electronics hardware, and software resident in Microsoft Excel® which lets us take and display data in real time and simultaneously control the instrument. In addition, it can post-process data, and even run the instrumentation remotely. This talk will present several different application examples. The modular software architecture makes it easy to mix and match tasks for whole new applications, and different instrument personalities. This has proven to be exceptionally powerful, economic, and useful, and saved us from spending tens of thousands of dollars for much more limited software and hardware.

Keywords: Automation, Materials Characterization, Process Analytical Chemistry, Separation Sciences
Application Code: General Interest
Methodology Code: Computers, Modeling and Simulation
Single-wall carbon nanotubes (SWCNTs) are an exceptional class of carbon-based nanomaterials produced in a variety of structural forms. Each distinctive structure possesses unique electronic, optical, and mechanical properties that make them attractive for various scientific and medical applications. However, to best realize these properties as produced mixtures must be sorted by their individual structures. Aqueous two-phase extraction (ATPE) is a technique recently demonstrated to successfully separate both surfactant-coated and DNA-wrapped SWCNTs by electronic structure, diameter, and helical handedness. The differential partitioning of the carbon nanotubes between the two aqueous polymer phases is governed by structure-dependent solvation free energy and can readily be tuned by number of parameters including temperature, dispersant composition, redox chemistry and the presence of salts. Additionally, ATPE is robust, cost-effective and does not require complicated instrumentation. Recent progress will be presented on the improvement, scalability, and automation of the ATPE method for SWCNT separation through the use of countercurrent chromatography.
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 ( and dispersed phase and the particles concentration ( ) according to the Mie theory:

\[ \text{BS} \text{ for Backscattering Intensity and T for Transmission Intensity.} \]

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 or PTA 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 paper, we present different complete studies:
- mean size measurement of various products, comparison of SMLS with SEM/TEM microscopy,
- dispersibility characterization of pigments,
- protein aggregation monitoring versus histidine concentration

**Keywords:** Characterization, Light Scattering, Particle Size and Distribution

**Application Code:** Nanotechnology

**Methodology Code:** Physical Measurements
A modular approach was developed to design a spectral library of core-shell nanoparticle contrast agents, which will have broad applications in biomedical imaging due to potential for multi-modal imaging (e.g., fluorescence, MRI, X-ray) and active targeting through molecular surface functionalization. Gadolinium oxide, hafnium oxide and gold core compositions were prepared at a common size (12-15 nm) using sol-gel and microemulsion syntheses. Nanoparticle cores were encapsulated in a silica shell with controlled thickness of 1-15 nm using polymer shells or Igepal. Controlled silica shell formation enabled the incorporation of fluorescent molecules and provided a common platform for molecular surface functionalization using silane chemistry. Antibodies and other small molecules were efficiently conjugated to the nanoparticles using EDC/NHS chemistry. The binding specificity of the NPs probes was then successfully tested against cancer cell lines associated with poor patient survival like SKOV3-IP, OVCAR3, OVCAR5, HCC1954 and MDA-MB-234. Statistical analysis was also done to estimate the binding kinetics of the NPs probes to the cell surface receptors and the correlation between NPs surface charge vs intra-cellular NPs distribution and intra-cellular NPs residence time. We anticipate this modular approach to provide a common platform for facile customization of core-shell nanoparticles for multi-modal imaging probes with tailored surface functionality.

Keywords: Biosensors, Nanotechnology, Protein, Single Molecule
Application Code: Nanotechnology
Methodology Code: Microscopy
### Session Title
Nanotechnology Applications

### Abstract Title
**A Nanoparticle Enhanced SPRi Platform for Multiplexed Analysis of Internal Organ Injury Biomarkers in Complex Matrices**

### Primary Author
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University of California Riverside

### Co-Author(s)
Quan Cheng, Samuel S. Hinman, Zhiguo Zhou

### Abstract Text
Major internal organ injuries can often lead to high morbidity and mortality rates, and the ability to identify this type of injury in a timely and sensitive manner is paramount to improving patient treatment. In particular, for trauma patients, the ability to assess multiple biomarkers for various organ injuries would increase diagnostic accuracy and allow for a rapid diagnosis. Surface plasmon resonance imaging (SPRi) allows for a real time observation of biomolecule interactions on an array of receptors, potentially allowing for multiplexed detection of a variety of biomarkers. Additionally, through the use of nanoparticle enhancement, sensitivity of detection can be achieved at the levels required for direct analysis of clinical samples. With nanoparticle enhanced SPRi, we have developed a method of analyzing a panel of biomarkers indicative of internal organ injury. This approach can be used for rapid analysis with minimal sample preparation, thus improving patient prognosis. This presentation will introduce a novel surface chemistry composed of a sacrificial membrane that can be prepared in an array format for analysis by SPRi, in an attempt to minimize nonspecific binding when carrying out analysis in crude serum samples. We will highlight the progress of our research, providing evidence of detection at clinically relevant concentrations in human serum, and discuss how this method of analysis will be extended to different organ markers for use in trauma patient screening.

### Keywords
- Bioanalytical
- Biosensors
- Nanotechnology
- Spectroscopy

### Application Code
Nanotechnology

### Methodology Code
Sensors
Acetylcholine is an essential neurotransmitter and neuromodulator of the nervous system. It is the most widespread excitatory neurotransmitter, utilized in both excitable and non-excitable cells. Current techniques, such as MRI, electrophysiology, and microelectrodes, have elucidated much about neural morphology and function. However, it is difficult to quantify both spatial and temporal fluctuations of acetylcholine signaling. One limitation in imaging these responses is the tight architecture of neurons and synapses. A tool for sensing acetylcholine in confined spaces would be beneficial for tracking the distinct molecular and neurophysiological mechanisms that govern complex neural pathways.

Previously, we developed a DNA-based nanosensor for sensing acetylcholine enzyme-based fluorescent system. We have advanced our technology for real-time imaging of acetylcholine dynamics and release in the retina. Here we show a tool for imaging the release of light-evoked acetylcholine in the retina. The nanosensor accommodates the enzyme and fluorophores in an optimal configuration for sensing; resulting in a brighter signal, targeting to cholinergic synapses, and improved sensitivity in the neurophysiological range with a lower limit of detection of 230nM. The DNA-based nanosensors are shaped in to stellar-dendrite structures with specific sites for conjugated sensing components. They are designed to take advantage of DNA's efficient self-assembling to maximally load sensing components that employ acetylcholinesterase as a recognition element alongside pH sensitive fluorescent reporters. This design is paired with tethered alpha-bungrotoxin to selectively immobilize the nanosensors to alpha7-nicotinic acetylcholine receptors of a postsynaptic neuron. Imaging the release of acetylcholine in the retina is done in Ambystoma mexicanum. We anticipate this work will further analytical capabilities of physiological processes in physically constrained environments.
Man-made nanoparticles (NPs) have found wide applications yet pose many potential health risks when interacting with environment and human bodies. 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 present study, conventional methodologies, cell viability, intracellular ROS accumulation, mitochondrial inner membrane potential, as well as the Apo-BrdU TUNEL apoptosis assay were employed and compared with our newly developed single cell micro-pH probe in detecting the early-onset of cytotoxic effects and cell deterioration process that caused by three types of NPs: silicon dioxide (SiO2), titanium dioxide (TiO2) and Cerium (IV) oxide (CeO2). Statistical data analysis of all conventional methods based populated cells was mostly incapable of showing significant cellular status variations when compared with control. However, the micro-pH probe from the single cell level can measure quick cellular responses to the NP-exposure mostly within 6 hours. This was evidently supported by the observed granulation phenomenon in the flow cytometry assay, and was further demonstrated in the TEM imaging of the NP-uptake cells. Even though the cytotoxicity of three types of NPs was not significantly different in conventional assays, large early-stage variations were actually revealed by our probe. In summary, the newly developed micro-pH probe showed superior capability in detecting true and early-onset of nanocytotoxic phenomenon through a real-time, staining-free manner on a single cell scale. This technique can potentially be used in re-evaluating the cytotoxicity of nanomaterials at the single cell level to unprecedentedly discover the true mechanism of nanotoxicity. Detailed experimental procedure and results will be presented at the conference. This work was supported by the National Institute of Health (1R21GM104696-01).
The transport of ions in semiconductor solids plays an important role in impurity doping, cation exchange transformations, and fast ion conduction. However, the thermodynamics and kinetics of ion transport is not fully understood, in particular, in nanoscale crystallites. Solid-state density functional theory (DFT) is being employed to understand the atomistic mechanism of cation incorporation, migration, and transport in chalcogenides. We are calculating the energies of formation of interstitial dopants and associated vacancies within lattices of the semiconductors cadmium, copper, and mercury selenide. In addition, energy barriers for the migration of such defects through the lattice are also being investigated. One of our primary interests is the determination the electronic structure origin of cooperativity seen experimentally in the transformation of cadmium selenide to copper selenide. A second topic of focus is the mechanism of super ion conduction in copper selenide. In the long run, we would also like to understand theoretically how crystallite size, strain, and surface energies dictate the energetics and kinetics of phenomena such as phase transitions and ion conduction. Such understanding will guide the design of nanoscale semiconductors for optoelectronics, batteries, and thermoelectrics and help uncover unique physical phenomena on the nanoscale.
Sample availability should be critically considered when developing novel bioanalytical methods. The use of small sample volumes has great advantages such as the reduction of both costs of analysis/disposal and the degree of invasiveness. Coated Blade Spray (CBS) is a SPME-based technology designed for the extraction/enrichment of analytes of interest from complex sample matrices, which can be directly coupled with mass spectrometry instruments for rapid screening/quantitative-analysis. In this study, CBS is introduced as an outstanding approach for the concomitant determination of therapeutic drugs and banned substances in blood and plasma spot samples. The whole analytical protocol consists of the following steps: a. extraction: spotting the biofluid onto the blade (V = 10µL); b. washing: fast removal of matrix constituents potentially adhered to the coated surface; and c. instrumental-analysis: spotting 10µL of the elution/electrospray solution (95/5/0.01 % methanol-water-formic acid) onto the CBS, which was placed 5 mm from the MS inlet. After 20s, a ± 5.5 kV potential difference was applied resulting in ESI from the tip of the blade. Due to the thickness and the biocompatibility of the coating (i.e. hydrophilic-lipophilic balance (HLB)-polyacrylonitrile (PAN) coating), fast extraction/enrichment (≤ 5 min) of the target analytes can be achieved with negligible adherence of matrix components onto the coated surface. Limits of quantitation investigated for a set of 15 drugs were found to be in the range between 1-10 ng mL⁻¹ in blood spots and between 1-5 ng mL⁻¹ in plasma spots. Great linearity in the range evaluated (i.e. 1-100 ng/mL), as well as precision (RSD ≤ 10%) and accuracy (88 - 120%) was estimated for three validation points (3, 40, 80 ng mL⁻¹). Analyte stability under different storage/transportation conditions was also studied.

**Keywords:** Bioanalytical, Clinical Chemistry, Mass Spectrometry, SPME

**Application Code:** Bioanalytical

**Methodology Code:** New Method
### Session Title
New Methods

### Abstract Title
A New Home-Made Generation System for VOC and Semi-VOC Gas Standards in Air

### Primary Author
Annarita Baldan

### Co-Author(s)
Dita Heikens, Hugo Ent, Janneke van Wijk, Stefan Persijn

### Abstract Text
Several VOCs (Volatile Organic Compounds) such as benzene and semi-VOCs such as phthalates are hazardous components affecting human health and therefore they are relevant pollutants in e.g. indoor air monitoring, emissions testing of building materials and in manufacturing environments.

VSL, the Metrology Institute of the Netherlands, is specialised in the production of traceable reference materials for VOCs and hold an ISO/IEC 17025:2005 accreditation as calibration laboratory.

Recently, VSL has built and validated a unique system based on a two-stage dynamic mixing of VOC and semi-VOC vapours with clean and dry air at atmospheric pressure conditions which allow achieving a concentration range down to 100 ppt (part-per-trillions). This system’s design is such that is preventing condensation of the vapours of the higher boiling point components such naphthalene, dibutyl phthalate and eicosane.

The gas standards obtained are used for direct calibration of analytical equipment, for active sampling of known amounts of VOC in sorbent tubes and for the Quality Control/Quality Assurance of exposure chambers.

The details of the design and the validation results will be addressed.

### Keywords
- Environmental/Air, Reference Material, Specialty Gas Analysis, Volatile Organic Compounds
- Quality/QA/QC
- New Method

### Application Code
Methodology Code

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**Date:** Thursday, March 09, 2017 - Afternoon  
**Time:** 02:30 PM  
**Room:** W176a
New Methods

**Novel High-Throughout Glass Surface Modification Method for Cell Attachment and Proliferation**

Surface functionalization of new materials suitable for cell attachment and proliferation has been a significant topic in biological studies for many decades. Specific surface chemical or morphological modifications are often required to provide sufficient hydrophilicity for cell attachment. Previous methods relied on either complex oxidative processes under exotic conditions or specialized structural and attachment proteins or polymers, such as Poly-D-Lysine (PDL). However, these methods are laborious, require complicated chemical modifications, suffer from poor reproducibility, and can pose safety hazards by transmitting dangerous biological agents. In this study, we have developed a novel and fast fabrication method to produce modified glass surfaces for cell attachment and proliferation by using a picosecond-laser (ps-laser). The resulting methodology is rapid (centimeter pattern scale in less than one minute) with high spatial resolution (5 – 10 micrometer) without complicated chemical derivatization. The data from the newly developed modified glass surfaces, conventional PDL-treated glass, and unmodified glass revealed superior cell attachment and proliferation in the newly developed method. In summary, this novel method presents a rapid and simple solution using ps-laser processing methods to fabricate modified glass surfaces for broad applications in the biological sciences. The detailed experimental procedure and results of this study will be presented at the conference.

**Keywords:** Biomedical, Laser, Material Science, Oxygenates

**Application Code:** Biomedical

**Methodology Code:** New Method
Evaluation of Different Extraction Methodologies for the Simultaneous Determination of Pesticides and Veterinary Drugs in Bovine Milk and Kidney

Nowadays the usage of phytosanitary products and veterinary drugs is a widespread technique in the livestock production chain. These contaminants could be retained in animal tissues and organs, contributing to the dietary exposure of the consumers. Every livestock producer should ensure food produced with acceptable levels of chemical residues. Therefore, the development of analytical methods for the simultaneous determination of pesticides and veterinary drugs residues in a modern way is crucial. Although there are plenty of methods reported for the analysis of pesticides and veterinary drugs separately, few of them have shown to be suitable for their simultaneous determination.

The group has previously validated an analytical methodology for the simultaneous determination of 56 pesticides and 21 veterinary drugs in bovine muscle and liver (cita). The main goal of this work was to check its applicability for kidney matrix and also to develop a methodology for milk. For kidney, the previously validated method for muscle presented good results according the SANTE/11945/2015 guidelines.

For milk 8 different extraction and clean-up strategies were compared through the percentage of recoveries and relative standard deviation. The methodology that presented the best results was chosen for validation according to SANTE. Although the chemical composition of the selected matrices (differences in fat, proteins, carbohydrates, and other metabolites content), is very complex, two straightforward methods based in a miniaturized solvent extraction followed by a dispersive purification step and finally, an HPLC or GC coupled with MS or MS/MS analysis, allowed the multiresidues determination, applicable to real samples.

**Keywords:** Food Safety, GC-MS, Liquid Chromatography/Mass Spectroscopy, Validation
**Application Code:** Food Safety
**Methodology Code:** New Method
New Methods

Enhanced Fluidity Liquid Chromatography Performed with Alcohol/Water/CO$_2$ Gradients

Supercritical fluid chromatography (SFC) has been boasted as a green technique that can yield rapid separations with comparable or better efficiency than the liquid chromatography counterpart. However, there is progress still to be made in regards to separating more polar analytes, which has been problematic for analytes that are only soluble in the most polar of solvents (i.e. water). Our group has proven that the use of enhanced fluidity liquid chromatography (EFLC) is a viable, green option for separating such polar analytes. EFLC is an alternative separation method that generally involves the use of a polar, organic/water mixture (a green solvent) as the primary mobile phase component and liquid CO$_2$ as the modifier in subcritical conditions. Commercial SFC instruments often only allow for gradients of a fixed organic/water composition for the mobile phase co-solvent. As such the polarity range (particularly the upper polarity limit) is restricted, and the ability to map CO$_2$ solubility (for maximum efficiency and resolution) over such gradients is limited. In this work, a modified commercial SFC instrument design will be presented that allows for variable modifier composition within a gradient and, with it, access to a wider polarity range. The effectiveness and necessity of this design will be demonstrated for the separation of biologically-relevant analytes such as peptides and carbohydrates.

Keywords: Chromatography, Instrumentation, Other Hyphenated Techniques, Supercritical Fluid Chromatography, Other

Application Code: Other

Methodology Code: New Method
Ultrafast lasers have been shown to be useful tools in increasing lateral resolution of mass spectrometry (MS) imaging. The <100 fs, 800 nm laser pulses effectively desorb neutrals from the sample surface with minimal fragmentation. It has been shown that the use of ultrafast lasers for MS imaging allows a lateral resolution of less than two micrometers on test samples (Y. Cui, et al., Anal. Chem. 2015: 87, 367). This technique is applied here to the analysis of lipids within intact slices of human pancreas tissue, with the ultimate goal of using single cell analysis for the study of diabetes. Experiments were run on a custom built reflectron time of flight designed to run laser desorption postionization (fs-LDPI) and laser desorption ionization (fs-LDI) MS experiments. A <100 fs, 800nm Ti:Sapphire laser was used for desorption cycling at 10Hz for fs-LDPI and 250Hz for fs-LDI. Postionization for fs-LDPI was achieved by ninth harmonic generation of a Nd:YAG laser to generate 10.5 eV (118 nm) vacuum ultraviolet pulses. MS images of fatty regions within the pancreas tissue were analyzed by both fs-LDPI-MS and fs-LDI-MS, in the former case without any addition of matrix. H&E stained optical images of the fatty regions of pancreas tissue were compared with the MS images of the same regions. Detection of lipids by fs-LDI-MS were compared to fs-LDPI-MS. It is shown that ultrafast lasers used for desorption can generate high resolution MS images of intact biological tissue without addition of any matrix.

Funding for this research provided by UIC.

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

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

**Methodology Code:** Surface Analysis/Imaging
Biomolecules at artificial surfaces play an important role in biomedical implants, biosensors, and pharmaceutical purification. Yet, oftentimes biomolecules that are active in solution lose activity when in contact with a support surface, due to perturbations in the folded state and conformational dynamics of the protein by the surface. Here, we image the heterogeneity of the folded state, stability, and folding dynamics of proteins at biomaterial interfaces using a novel technique, Fast Relaxation Imaging (FReI). FReI has been previously used in cellular environments, yet has untapped potential to understand proteins in complex material environments. We resolve the heterogeneous folding and stability of fluorescently-labeled phosphoglycerate kinase within polyacrylamide hydrogels, an important material for protein separations and extracellular matrix supports. The cross-linking density of the material was varied, showing an optimal steric crowding effect where the excluded volume stabilizes the protein but the polymer is not too dense to interact and disrupt the structure of the protein. Overall, the achievement of FReI at material surfaces engenders a new imaging approach that identifies the impact of local protein-material interactions on protein stability. This could direct design rules for the engineering of biomedical devices and materials, improving the performance, shelf life, and cost.
Understanding interactions of DNA with chemically modified surfaces is of great importance in DNA microarrays, advanced biosensors, separation systems and new gene delivery materials. Development of optimal materials for the aforementioned applications usually requires preparation of a large combinatorial library of materials incorporating different surface functional groups of varying densities. Chemical gradients afford a means to avoid this tedious process, allowing for a continuous range of materials properties to be explored along a single substrate. For studying interactions of DNA with chemically modified surfaces, single molecule detection not only directly visualizes dye labeled single DNA molecules, but their dynamic behaviors can also be explored in real time. Moreover, electric fields play an important role in DNA detection but their influence on the interactions of DNA with chemically modified surfaces has not been widely studied. Thus, in this report, interactions of YOYO-1 dye labeled plasmid DNA with 3-aminopropyltrimethoxysilane (APTMS) gradient modified indium tin oxide (ITO) electrodes are investigated as a function of amine coverage, charge state, and applied potential using single molecule detection methods. APTMS gradients are characterized by water contact angle measurements, ellipsometric measurements and X-ray photoelectron spectroscopy to test their hydrophobicity, thickness variation and chemical composition. From the optical results, it was found that plasmid DNA can only be captured and released at low amine coverage and high pH, when positive and negative potentials are applied, respectively. At high amine coverage, permanent adsorption of the DNA occurs at both low and high pH due to electrostatic and hydrophobic interactions.

Keywords: Adsorption, Chemically Modified Electrodes, Microspectroscopy, Single Molecule
Application Code: Bioanalytical
Methodology Code: Microscopy
Chemical and physical interactions play important roles in surface film formation and fluid slip at the fluid-solid interface. To investigate a fluid’s transition between bulk and interfacial, a dynamic wetting technique is combined with vibrational spectroscopy and ellipsometry to allow direct investigation fluid films of variable thicknesses that span the bulk to interfacial transition. The dynamic wetting technique utilizes a vertically aligned, disk-shaped substrate that rotates through a bulk fluid droplet, held at the lower half of the substrate by a glass capillary. As the substrate passes through the droplet, a thin fluid film is extruded and ultimately probed at the apex of the rotating surface in a reflection spectroscopy geometry. The thickness of the fluid film is systematically varied by controlling the velocity of the substrate rotation. Our results show that the structure and orientation of fluid molecules within these films are highly dependent on the film’s thickness. Specifically, acetophenone film thicknesses are linearly related to substrate velocity^(2/3) but are substantially offset from the absolute thickness values. Results also inform ongoing studies to examine the thin film behavior for a series of tri-alkylamines. Preliminary results show significant changes in the vibrational profile as a function of film thicknesses. These are ascribed to increased intermolecular forces and fit with the generally accepted model of increased levels of fluid intermolecular structuring with proximity to a solid surface.
This work demonstrates the usefulness of SHG imaging to quantify crystallinity content in a large dynamic range of dosage forms without the use of a calibration plot. The pharmaceutical industry increasingly uses amorphous forms of active pharmaceutical ingredients (APIs) to improve solubility and dissolution. Detection of crystallinity in amorphous formulations is critical for the assessment of the safety, stability, and efficacy of APIs. Second harmonic generation imaging has proven to be a successful method for the detection of trace crystallinity within amorphous systems. However, despite the success of the low limit of detection, precise quantification of residual crystallinity by SHG continuously spanning a wide dynamic range of crystalline content remains challenging. This work demonstrates how the low and high crystallinity regimes can be merged through a statistical model based on analytical expressions for the optimization of signal to noise. The authors acknowledge support from the GOALI NSF program.
Elemental impurities are metals that may occur naturally in a pharmaceutical process, are added intentionally via use of metal catalysts or reagents, or can be inadvertently introduced through interaction with processing equipment. Understanding the fate of these metals and controlling them to appropriate limits is a regulatory expectation. Inductively coupled plasma optical emission (ICP-OES) and/or mass spectrometry (ICP-MS) are the traditional analytical techniques for monitoring elemental impurities, but in a continuous processing environment, ICP laboratories can struggle to manage the large number of samples and fast turnaround time required to enable continuous process decision making. X-ray fluorescence (XRF) is a quick, simple, and selective technique that can be used to gather elemental impurity data throughout a continuous process and can provide data that supports the rejection of specific metals or lack thereof, within a matter of minutes. The focus of this presentation will describe how XRF was applied to support continuous processes and show that it is a viable alternative to ICP through a direct comparison of data generated by both XRF and ICP.

Keywords: Elemental Analysis, ICP, Process Monitoring, X-ray Fluorescence
Application Code: Pharmaceutical
Methodology Code: X-ray Techniques
Atomic structures of the superionic solid Cu[sub]2[/sub]Se have been studied in detail in the bulk. At room temperature, Cu[sup]+[/sup] ions and vacancies form an ordered super-lattice. Above 400 K, the Cu[sup]+[/sup] sub-lattice structure becomes disordered and “liquid-like” giving rise to a solid that has super-ionic conductivity, with applications in solid-state electrolytes and fuel cells. However, this order-disorder transition behavior may be quite different in nanocrystalline Cu[sub]2[/sub]Se due to surface effects. However, the crystal structure in nanosized crystallites of Cu[sub]2[/sub]Se is not fully understood. Furthermore, the atomistic dynamics of the order-disorder phase transition of Cu[sup]+[/sup] ions and vacancies is not known.

We are studying using in-situ transmission electron microscopy (TEM) the atomistic structure of Cu[sub]2[/sub]Se and the dynamics of the non-superionic to superionic phase transition in ca. 10 nm sized nanocrystals. Using millisecond-imaging combined with structural simulations, we are elucidating the nature of atomic rearrangements that occur in the course of the phase transition. In addition, the atomic-resolution structure of Cu[sub]2[/sub]Se in the high temperature phase will allow us to understand the pathways and mechanisms for Cu[sup]+[/sup] super-ionic conduction in nanocrystals.

Keywords: Materials Characterization, Material Science, Microscopy, Nanotechnology
Application Code: Material Science
Methodology Code: Microscopy
The exposed surfaces of man-made structures represent ubiquitous substrates for the deposition and reaction of atmospheric persistent organic pollutants (POP). It is known that 'urban grime' films accumulate on these surfaces and contribute significantly to fate and transport of POP. The characterization of these surface films is urgently needed to better define the POP / surface interactions. Urban films are exposed to a wide range of environments and maturation agents, i.e. temperature swings, exposure to UV-radiation and ozone, and varying amounts of rainfall. Mapping the physical and chemical morphology of these systems will provide insight into the controlling factors in their development and maturation. These measurements are critical to improve predictions and knowledge of the fate and transport for POP and other atmospheric constituents. This talk will focus on quantifying the physical properties of native urban films and creating suitable model systems to replicate their chemistry and morphology for laboratory analyses. We present microscopy, spectroscopy, and QCM-absorption data on a series of native films collected over >12 months, as well as preliminary data from a laboratory-based model of an urban film.

Keywords: Environmental Analysis, PAH, PCB's, Spectroscopy
Application Code: Environmental
Methodology Code: Surface Analysis/Imaging
In the case of Ionic Liquids (IL), surface-induced molecular ordering has been reported up to extend up to 50 nm. We use a dynamic wetting technique to make IL films of variable thickness on silver surfaces. Spectroscopic analysis provides evidence of very long range ordering; up to several thousand nanometers from the solid surface. We describe this ordering by analysis of infrared and second harmonic generation measurements. Large changes are observed in the IR absorption profiles for the low frequency anion peaks (900 cm⁻¹ to 1400 cm⁻¹). These are distinct from the simple freezing transition, and indicate a clear orientation change of the IL molecules. These changes occur over timescales of several minutes to hours. Additional measurements of SHG response of the films show large increases over similar timescales, which is also indicative of increased molecular ordering in the film. The data suggests a slow but spontaneous ordering effect that permeates the film’s entire thickness. The ordering transition is completely reversible, and results are presented for varying surface chemistries, viscosities, and varying IL classes, with direct comparison to temperature controlled, transmission FTIR data, acquired at temperatures correlated to bulk phase transitions as obtained from differential scanning calorimetry.

Organic Modified Silane (ORMSIL) compounds are tetra-functionalized organic-inorganic complexes containing three alkoxy groups and a fourth organic functionality all bound to a central silicon atom. One of the major applications for ORMSIL chemistry is surface functionalization (Figure 1). When covalently bonded onto glass, or another silica-based surface, ORMSILs may allow tenability of the physical and chemical properties of the surface. With ORMSILs, hydroxyl-containing inorganic surfaces can be specifically and deliberately tailored to perform a variety of functions including sensing, super-repellency, and biomedical processes. This talk will detail how the ORMSILs chemistry allows fabrication of the super-hydrophobic and oleophobic surfaces as well as sensors for analytical detection. Structural Hierarchical Modified Microsphere Surfaces (SHiMMS) have been fabricated using ORMSILs chemistry that yield super-hydrophobicity and oleophobicity to the substrates, with an average static contact angle of water in a range of 150 ±9° and~132+5°, respectively (Figure 1A). Dynamically the SHiMMS will either roll off the surface or remain pinned depending on the kinetic energy of the impinging drop and the surface roughness of the SHiMMS. The micro-roughness of the SHiMMS can also be used to encapsulate aqueous media (i.e. water), which is reminiscent of a cell-lipid bilayer membrane at micrometer and millimeter scales and allows for the fabrication of highly spherical water droplets on a variety of surfaces (Figure 1B). Functionalization of these silica micro particles with the ethylenediamine-tetraacetic acid (EDTA) ligand affords an inexpensive and facile filtration device for the removal of heavy metals from water. The application of ORMSILs chemistry to surface science may provide inexpensive and readily available materials composed with locally available materials.
Self-assembled monolayers (SAMs) of derived silanes are extensively used for surface functionalization. Special experimental conditions are essential for a successful surface silanization. Moreover, many silane derivatives are sensitive to water hydrolysis and therefore require storage in inert gas atmosphere increasing labor and material cost of functionalization. Siloxanes oligomers and polymers are chemically stable in ambient conditions. They were considered as “unreactive” to most surfaces over past 50 years until McCarthy et. al. found that liquid-phase poly(dimethylsiloxane) (PDMS) can covalently bind to inorganic surfaces via a water-assisted process. We report here Siloxane-bound Layers through Vapor-Enhanced Deposition (SOLVED) where the siloxanes were covalently bound to both the inorganic and organic surfaces. We found that a self-limiting ~6.5 nm thick siloxane layer was covalently bound to glass using a thermal vapor deposition temperature of ~150-250°C. Our FTIR and X-ray photoelectron spectroscopy (XPS) results clearly show that the PDMS, amino-co-polysiloxane (A-PDMS) and fluoro-polysiloxane (F-PDMS) were successfully deposited on glass, aluminum, stainless steel, and polyethyleneterephthalate surfaces while maintaining their primary chemistry. The siloxane binding mechanism and kinetics to the surface will also be discussed in this talk. Our cyclic voltammetry (CV) studies indicate that the siloxane-bound layers are defect-free and possess similar UV stability to that of highly stable SAMs. Finally, we will demonstrate enhanced resolution imaging using micro-lens (MLs) array fabricated on the SOLVED surfaces. SOLVED is believed to be a powerful method for a one step and easy to accomplish robust method for the surface covalent modification and may find many potential application in the materials- and life-sciences.

Keywords: Membrane, Microscopy, Surface Analysis, Thermal Desorption
Application Code: Material Science
Methodology Code: Surface Analysis/Imaging
Confocal Raman imaging is a powerful tool for sample characterization. In this measuring technique an extremely sensitive confocal microscope is combined with an ultrahigh throughput spectroscopy system, enabling the acquisition of ten-thousands of Raman spectra with integration times in the millisecond range. The result of such measurements is a chemical/molecular identification of spices within a sample with true diffraction limited resolution. The power of the Raman imaging technique is reflected in more than 500 papers published during the past years. The new generation of Raman imaging systems relays in the automation routines for an easy workflow of the experiments: change of wavelength, adjustment of the confocal pinhole and entrance slit for the spectrometer, change of laser power to avoid sample damaged are all included in these routines. The aim of this contribution is to highlight the benefit of the new features of an automated confocal Raman imaging system together with examples from various fields of applications.

Keywords: Infrared and Raman, Material Science, Microscopy
Application Code: Material Science
Methodology Code: Surface Analysis/Imaging
Well-ordered monodisperse, nanoparticle monolayers are utilized in many fields including sensing materials, photonics, electronics, nanosphere lithography, and photovoltaics. We previously developed a facile method for producing large area highly ordered hexagonally close packed monolayers of particles using a needle tip flow technique. The highly negatively charged polystyrene nanospheres self-assemble into a 2D Photonic Crystal (2DPC) at an air/water interface. We investigate the effect of the particle size and interparticle electrostatic interactions on the 2DPC ordering. Negatively charged (zeta potential -50 mV) monodisperse, polystyrene nanospheres with diameters of 413, 570, and 915 nm were examined. The nanoparticles were self-assembled on surfaces of aqueous solutions of different salt concentrations. The increasing ionic strengths increasingly screen the electrostatic interactions between nanoparticles. We observed that self-assembly of ordered 2DPC requires strong electrostatic repulsions between particles. As ionic strength of the water increases slightly the crystallite domain size decreases, further increase of the ionic strength results in a loss of long range ordering all together. Ordering was measured by calculating the 2D pair correlation function (g(r)) from SEM images of the self-assembled 2DPC. The Fourier transform of the function g(r)-1 of the fabricated 2DPC was compared to that of a perfect array to quantitate ordering, then compared to the diffraction pattern of the Debye rings. We observe that the width of the Debye ring quantitatively depends on the 2DPC ordering.

Keywords: Light Scattering, Materials Characterization, Material Science, Nanotechnology
Application Code: Nanotechnology
Methodology Code: Surface Analysis/Imaging
Surface Modification/Imaging Developments

Assembling Nanoparticle Contrast Agents for Spectral (Color) X-Ray Imaging

Recent advances in energy-sensitive X-ray detectors has enabled spectral (color) computed tomography (CT) and the potential for molecular imaging in CT. However, the X-ray attenuation of soft tissues requires the use of contrast agents to achieve sufficient spectral contrast for imaging. Therefore, objective of this study was to demonstrate the ability of spectral CT to simultaneously detect up to three different core-shell nanoparticle (15-20 nm) contrast agents, comprising gadolinium oxide, hafnium oxide and gold core compositions, exhibiting K-edges spaced 10-20 keV apart, by spectral unmixing. The contrast agents were imaged in a soft tissue equivalent phantom using both source-side (monochromatic synchrotron radiation] and detector-side (photon-counting detector) methods for spectral CT. The ability to detect and distinguish the X-ray contrast agent from surrounding soft tissue was then demonstrated in a mouse model. We also demonstrated the ability to control the thickness of the silcia shell on the different cores and to control the distribution of surface functional groups on the core-shell nanoparticles to modulate their payload capacity and biocompatibility. The nanoparticles designed for this study have broad applications in biomedical imaging due to their potential for multi-modal detection (MRI, fluorescence, plasmonics) and surface functionalization with biomolecules for active targeting and molecular imaging.

Keywords: Imaging, Immunoassay, Modified Silica, Nanotechnology
Application Code: Material Science
Methodology Code: X-ray Techniques
### Abstract Title
Sub-Molecular Resolution Interrogation of a Surface-Mediated Conformational Switch by Ultrahigh Vacuum Tip-Enhanced Raman Spectroscopy

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### Abstract Text
Tip-enhanced Raman spectroscopy (TERS) combines the ability of scanning tunneling microscopy (STM) to resolve atomic scale surface features with the single molecule chemical sensitivity of surface-enhanced Raman spectroscopy (SERS). The goal is to understand and manipulate chemistry on the nanometer length scale. Here, new insights into the nature of a conformational dynamics involved at room temperature will be presented. We have interrogated the conformational change of meso-tetrakis-(3,5-di-tertiarybutylphenyl)-porphyrin (H2TBPP) on a Cu(111) surface between two stable conformations. At room temperature, the barrier between the porphyrin ring buckling up/down conformations of the H2TBPP-Cu(111) system is easily overcome, and our group has achieved unprecedented sub-nm resolution by simultaneous UHV-TERS and STM analysis. This topic illuminates that TERS can unambiguously distinguish the conformational differences between neighboring molecules with single molecule resolution. Furthermore, the sub-nm resolution led to the direct observation of single molecule transitions between states from one scan to the next.

### Keywords
Imaging, Nanotechnology, Raman Spectroscopy, Vibrational Spectroscopy

### Application Code
Nanotechnology

### Methodology Code
Surface Analysis/Imaging
RISE Microscopy is a novel correlative microscopy technique which combines confocal Raman Imaging and Scanning Electron (RISE) Microscopy within one integrated microscope system. This unique combination provides advantages for the microscope user with regard to comprehensive sample characterization: electron microscopy is an excellent technique for visualizing the sample surface structures in the nanometer range; confocal Raman imaging is an established spectroscopic imaging method used for the detection of the chemical and molecular components of a sample with diffraction limited resolution. In contrast to existing combinations, where single Raman spectra are typically collected from few micrometer size areas, the RISE combination allows for the first time diffraction limited confocal Raman imaging on the same sample position as the SEM image was taken. It can also generate 3D-images and depth profiles to visualize the distribution of the molecular compounds within a sample volume. Both analytical methods are fully integrated into the RISE Microscope. Between the different measurements a precise scan stage automatically transfers the sample inside the microscope's vacuum chamber and re-positions it. The integrated RISE software carries out the required parameter adjustments and instrument alignments. The acquired results can then be correlated and the Raman and SEM images overlaid. The instrument as well as various examples for using this new possibility for correlative confocal Raman imaging with SEM will be presented.

Keywords: Instrumentation, Materials Characterization, Microscopy
Application Code: Nanotechnology
Methodology Code: Surface Analysis/Imaging