

Meeting Abstracts

Abstracts of Papers Presented at the 2007 Pittsburgh Conference

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AUTOMATED TRACE EXPLOSIVE EXTRACTION SYSTEM FOR AIRPORT LUGGAGE

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A system has been designed which is based on an adaptive, flexible, glove-like membrane that forms a smaller volume and hermetic enclosure around the inspected item (checked or carryon luggage), with minimal stray air around it. This minimizes the air quantity that needs to be treated, manipulated, and examined, while increasing the “signal-to-noise” ratio. Clean air is introduced in the enclosure by pulsed jet nozzles while the air is collected by special suction nozzles. The blowing and suction nozzles are mounted on the membrane, with maximum efficiency due to proximity to the inspected surface. Several compression/decompression cycles allow extraction of air carrying traces of explosives from inside the inspected item. The air is then passed through an electrostatic trap to collect these particles and pass them finally to a trace analyzer, such as an ion mobility spectrometer (IMS). The automated trace collection is effected without manual intervention, using a device that collects the particles using pneumatic methods and mechanical agitation.

Detection time is below 30 seconds/per baggage. The system allows for remote standoff operation, decreasing the risk of blast to personnel, whilst the false positive alarm rate is not more than the false alarm rate of the sensor system. This is based on testing conducted at Tel Aviv’s Ben Gurion International Airport, where over 1000 pieces of hand luggage were tested using a Smith’s Detection Ionscan 400 ETD.

OPTIMIZING SYSTEM PERFORMANCE IN HAND-HELD MINE DETECTION

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Effective and safe hand-held mine detection is dependent on optimizing the man-machine interface. For this to happen the operator must be optimally trained while the detector must be designed to be easy to use. That means it must be simple to operate in a manner that the human is capable of doing within a practical amount of training, and the information provided by the detector must be reliably accurate and easy for the operator to understand. For humanitarian deminers, training and many hours of use may eventually enable fitting the human to the machine, assuming a mistake is not first made. But for soldiers, who have many other tasks in which they must also continuously maintain skills, the design of both the detector and the training task must be as simple as can be safe and effective.

The Army Research Laboratory in collaboration with Carnegie Mellon University, the University of Missouri-Rolla, and Lincoln University has conducted various research studies pertaining to hand-held mine detection. The results of these studies are providing guidance for improved detector design, improved training techniques, and better training tools and facilities. The objectives of these studies have been to optimize soldier performance with the detector.

These studies have focused on methods for rejecting clutter, influence of distracters on probability of detection, learning decay over one- to three-month intervals, effectiveness of computer-based instruction, and potential for supplementing detection with visual techniques.

This presentation will summarize the findings from these studies and present recommendations for improving Soldier's hand-held mine detection performance.

OPTIMIZING THE IMMOBILIZATION OF BOVINE PULMONARY ARTERY ENDOTHELIAL CELLS INTO POLY(DIMETHYLSILOXANE) MICROCHANNELS

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A microfluidic-based circulatory vessel comprised of bovine pulmonary artery endothelial cells (bPAECs) within poly(dimethylsiloxane) PDMS-based channels is described. Here, the optimal concentration, incubation temperature, and period of incubation time for fibronectin and bPAECs, two components of the immobilization, are described. To optimize the number of viable cells adhered to the channels, varying concentrations of fibronectin ranging from 1 $\mu\text{g}/\text{mL}$ to 500 $\mu\text{g}/\text{mL}$ were incubated in a PDMS-coated 24-well sterile plate, aspirated off, and a cell suspension (>50,000 cells/mL) was added to each well. The incubation time (0 to 90 minutes) and temperature (25°C to 37°C) were also examined in a similar manner. For bPAECs, the optimal period of cell incubation on fibronectin-coated PDMS and the appropriate density of cell suspension for confluency were established. Results suggest that fibronectin concentration, as well as incubation temperature, impacts the number and viability of cells that adhere to the PDMS surface. Fibronectin concentration was determined to be optimal at 50 $\mu\text{g}/\text{mL}$ and a 10% increase in the number of viable cells was observed when the incubation temperature was raised to 37°C. A correlation exists between the number of viable cells on the PDMS and an increase in the incubation time of the cells, as well as an increase in bPAEC density. The optimal bPAEC incubation time was determined to be 120 minutes with the bPAEC solution having the highest density (150,000 cells/mL) becoming confluent in the well plate within a few hours, while dilutions of the solution were still not confluent after an overnight incubation. The incubation time of fibronectin has little effect on the average number of cells that grow on the plate after 15 minutes. These results were then applied to create a more reproducible biomimetic system using microfluidic technology for studying multiple cell lines in a microchannel.

A MICROFLUIDIC DEVICE FOR LONG-TERM CHEMICAL MONITORING OF LIVING CELLS

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Microfluidic devices are valuable tools for studying cells and are potentially compatible with long-term cellular measurements. It is possible to integrate and automate several functions on one chip, including sample collection from cells,

injection of the sample, and detection of analytes. Furthermore, it is simple to manipulate the cellular environment in a microfluidic space, allowing for good temporal resolution for monitoring. Previously, we developed a device that can maintain living cells in culture and monitor their secretion at 6-second intervals by an electrophoresis-based immunoassay. The device has been applied to monitoring insulin secretion from single islets of Langerhans over periods of 2 hours. In the present work, we describe developments that allow this device to be used for monitoring over periods of 1–5 days. Previously, long-term continuous monitoring on the chip was limited by degradation of reagents and electrophoresis buffers. To resolve this problem, in the modified chip, all reagents were continuously perfused into the device with a gas pressure system, so only fresh reagents were used for analysis. The introduction of particulates, which clog the microfluidic channels, prevented long-term immunoassay stability. The source of the particulates was determined to be a component of the gas pressure system and was then replaced. Failure of the electrical relay, which controlled the sample injection, also hindered the immunoassay stability; this problem was solved by using a relay rated for longer operation (1×10^9 cycles). With these modifications, the immunoassay had a 4% RSD over 24 hours of continuous operation and a detection limit of 0.9 nM. The long-term monitoring chip will now be used for novel experiments that require measurements of slowly developing phenomena of islets such as temporal changes that result from time in culture and effects of lipids.

ON-SITE SPOT SAMPLING OF WATER USING SPME FIBERS AND PDMS THIN FILMS WITH A CONTROLLED AGITATION TECHNIQUE

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Field sampling is often influenced by many environmental variables such as temperature, turbidity, and water flow. When using SPME for field sampling, many of these variables can be eliminated or minimized. SPME extraction is dependent upon extraction conditions such as temperature and agitation. Temperature is not possible to be controlled in the field but we can control the agitation of the fiber in the water sample. Once the agitation is controlled, the concentration of analytes can be quantitated because the thickness of the boundary layer, a zone of defined width between the fiber and the bulk of the fluid where no convection occurs, can be estimated based on empirical formulae. Portable hand-held stirring devices have been developed using cordless drills. A special "drill bit" was created to hold an SPME fiber or a piece of PDMS thin film which are the two extraction techniques used in our experiments. Comparisons between the agitated fiber, the agitated thin film, the static fiber, and the static thin film were completed and showed that the agitated thin film has the fastest extraction time and the largest capacity. This is due to the high surface-to-volume ratio of the thin film

compared to the SPME fiber. We are able to achieve rapid, reproducible extraction on site using the hand-held stirring device with very little cost.

A FULLY VALIDATED PASSIVE SAMPLING METHOD FOR AIRBORNE LOW-MOLECULAR ISOCYANATES

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Isocyanates are important compounds in the field of occupational hygiene. They show severe acute toxicity and have also strong sensitizing properties. Sensitization usually takes at least several weeks of exposure, but then even very low concentrations below current occupational exposure limits (OELs) could trigger life-threatening asthma attacks.

As for the determination of isocyanate concentrations in air, all routine and standard measurements are based on active sampling methods. In recent years, passive sampling methods have proven great effectiveness and reliability for air monitoring applications for many airborne analytes. Regarding isocyanates, there are up to now only two fully validated passive methods for methyl isocyanate (MIC) known from the literature [1, 2], as well as an early approach [3] reporting a passive dosimeter for toluene diisocyanate (TDI). Furthermore, a method for diffusion-controlled sampling of TDI and hexamethylene diisocyanate (HDI) using SPME devices has been reported.

In this paper, we present a fully validated diffusive sampling method for the determination of airborne methyl (MIC), ethyl (EIC), and phenyl (PhIC) isocyanates using 4-nitro-7-piperazinobenzo-2-oxa-1,3-diazole (NBDPZ)-coated [4] SDB filter tapes as collection material. Validation was carried out by active sampling using 1-(2-methoxyphenyl) piperazine (2-MP) as derivatizing agent. Sampling intervals were between 15 minutes and more than 8 hours. Sampling rates ranged from 21.0 mL/min for MIC down to 11.5 mL/min for PhIC. The limits of quantification were 1.4 ppb for MIC and 1.3 ppb for EIC and PhIC applying 15-minutes sampling periods.

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LOW-COST HAND-HELD AND ONLINE SPECTRAL MEASUREMENT SYSTEMS FOR ENVIRONMENTAL MONITORING APPLICATIONS

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In 2004, EPA warned against widespread pollution of lakes and rivers by mercury, dioxin, PCBs, pesticide pollution and other contaminants. The EPA administrator confirmed that nearly every time officials check for pollution, they find it. The Natural Resources Defense Council reported in 2005 that unknown and illicit sewage sources continue to degrade natural habitats and thereby increasing the risk of infectious bacteriological diseases in drinking and recreational waters resulting in 19 950 beach closing/advisory days in 2004—a tenfold increase since 1994.

Keeping the nation's water supply clean and safe is of increasing concern, and is compounded by the inability to trace illicit contamination sources. EPA is identifying endocrine disruptors from 87 000 chemicals. Emerging contaminants enter waterways through the 90 000 municipal, industrial, pharmaceutical, and agricultural wastewater systems.

Over the next decade, it has been suggested that potable water resources, on a worldwide basis, will be a more important issue than the current concerns about fossil fuels. Taking into account all of the above comments, it is becoming increasingly important to have easily deployable instrumentation and sensing systems for the monitoring of all aspects of water quality.

MicroSpectral Sensors has developed a new miniature, multiparameter spectral measurement system as a low-cost solution for field-based and online monitoring of environmental contamination threats. These are intended to provide real-time measurements of a wide variety of contaminants. This paper will review the applications of this new technology to current water-quality measurement methods.

A NEW APPROACH TO LABORATORY INFORMATICS

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A laboratory uses a range of informatics components including LIMS, CDS, ELN, and ECM/SDMS. The role of each of these components overlaps and it is not always clear which solution is the best. For example, which system do you use to store chromatography results?

Even though these better-known systems handle a lot of the laboratories' requirements, there are still key areas that

until recently have no clear solution. Managing manual procedures and automating small instruments are two key areas.

Other than specific instrument data systems, the common informatics products available today are not designed for real-time management. They play a role in planning the work for the laboratory and in storing results, but they do not get involved in the actual analysis process. Consequently, their ability to manage workflow and control analysis is limited or nonexistent. Real-time management of the laboratory requires a completely new approach.

This presentation will examine the role of each of the common informatics components discussing both their strengths and weaknesses. It will then highlight key areas that are still lacking automation solutions. Finally, it will present a new concept in informatics that integrates the strengths of existing components with new real-time management control capabilities. This new concept redefines the role of informatics in the laboratory.

ELECTRONIC LABORATORY NOTEBOOKS FOR ROUTINE PROCEDURES

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Most electronic laboratory notebooks have been designed to assist in R&D operations. Many of the key considerations for these ELNs are based on concepts involving collaboration, documentation, and protection of intellectual property. This type of design however does not lend itself well to the requirements of automating routine procedures. For routine analyses, there is a need to control the information that is recorded in order to ensure that it fulfills requirements and follows standards. Unlike the R&D environment, it is not acceptable or desirable to record any observation or any data that the analyst feels is appropriate. Instead, the requirement is for a much more controlled set of data.

In this session, we will highlight key considerations when investigating an ELN for routine procedures and discuss the benefits that can be realized by utilizing this technology as part of a laboratory informatics strategy.

CONNECTING THE LABORATORY TO THE ENTERPRISE USING WEB SERVICES

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One of the key problems laboratories are trying to solve is how I make my results available to the people who need them. How can I help them to make decisions? Laboratories can have hundreds of different instrument and equipment types from different vendors all with different software packages. This talk will discuss how use of web services and XML can simplify this process for the laboratory and ensure quick decisions for the business.

FAST TRACK LIMS

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What is special about fast track LIMS? Rigid adherence to principles of “fast” and “On track” phased implementation focused on achievable, priority deliverables, rapid decision making, constant monitoring and communication of progress against deliverables and resolution of issues, and so forth.

Laboratory information management systems (LIMS's) are highly complex systems that support multiple users and business processes that cross organization boundaries. LIMS interfaces with a myriad of instruments systems for data collection and helps achieve business goals through integration with other business systems. Implementation of LIMS is difficult. The road is littered with LIMS projects that did not quite meet their intended goals. Projects are frequently late, run over budget, or stop short of producing the results that were used to justify the original project.

Fast track LIMS is a proven methodology that gets LIMS implementation done quickly and keeps it “on track.” “On track” means that the implementation focuses on the correct end goals, is accomplished with efficient use of both time and resources, and produces an enduring solution that meets business needs.

This presentation will describe the principles and activities involved in the fast track methodology. The paper will address the full LIMS life cycle starting with requirements, vendor selection, design and build, configuration, and data loading through going live. The speakers will discuss some of the typical pitfalls awaiting a LIMS implementation and describe keys to success or proven ways of addressing these pitfalls. A case study will be presented highlighting the critical decisions that enabled fast track implementation and reviewing barriers to optimal execution.

AUTOMATING CGMP QC LABORATORIES USING A GMP ELECTRONIC NOTEBOOK SYSTEM

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Pharmaceutical R&D and manufacturing have not changed their fundamental paper-based infrastructure in decades. The principal reason is that science by nature and regulatory requirements resist change. Recently, industry and FDA have been more aligned with respect to utilizing innovation and technology to bring manufacturing processes into the 21st century. Leading companies are adopting a new approach to “automating R&D and compliance,” that is, utilizing innovative technologies (such as ELN strategies) and building quality into the research, quality, and compliance infrastructures.

The talk will discuss the current situation in cGMP lab operations relative to FDA initiatives in manufacturing and the broad opportunity for electronic lab notebook processes. The paper will profile an innovative “method-centric” software platform, designed to electronically execute and manage laboratory protocols, yielding significant reductions in overall method execution cycle times, and electronically capture all data and metadata in a common repository. Several large pharmaceutical IT infrastructure design examples will be outlined.

EASING SOFTWARE VALIDATION AND IMPLEMENTATION THROUGH THE DEPLOYMENT OF A MODERN SOFTWARE FRAMEWORK

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One of the most onerous tasks for a regulated lab is simply taking advantage of their investment. The cost and effort of validating and implementing laboratory software have forced laboratories to upgrade only every three to five years to ensure a positive return on investment. By using new software technology, vendors can deliver software that is easier to implement and validate and provides a real benefit to the end users.

SIMULTANEOUS COEXTRACTION OF MULTISPECIES OF DIFFERENT ELEMENTS FROM BIOLOGICAL SAMPLES BY CLOSED-VESSEL MICROWAVE EXTRACTION AND DETERMINATION BY INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY COUPLED TO LIQUID AND GAS CHROMATOGRAPHY

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Organometallic species of mercury, lead and tin, and redox states of chromium play important roles in biological as well as environmental processes because they are highly toxic and can be of anthropogenic and/or biogenic origin. A simple and efficient coextraction method was developed for simultaneous extraction of different species of mercury, lead, organotins, and chromium from biological samples by using closed-vessel microwave-assisted extraction protocol. The developed extraction method was validated using certified reference materials of biological origin and applied to biological samples. The developed extraction method was validated by implementing EPA method 6800 as a diagnostic tool for species transformation during extraction and/or analysis using ICP-MS coupled with both liquid and gas chromatography. This coextraction method for multispecies analysis incorporates many benefits of microwave extraction and method 6800 in terms of speed, low solvent use, accuracy of measurement, sensitivity, relative simplicity, as well

as the time saving and convenience of multiple species measurement through sample preparation and analysis as an integrated step.

AUTOMATED UPLC METHODS DEVELOPMENT: MONITORING SELECTIVITY CHANGE BY CONTROLLING STATIONARY PHASE, ORGANIC MODIFIER, PH, AND TEMPERATURE

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UPLC allows chromatographers to get higher-resolution, faster chromatographic methods by improving system efficiency. In addition to improved efficiency, chemical factors can be manipulated to improve the selectivity and retentivity of a separation. In this lecture, we explore and measure how factors such as pH, organic modifier, temperature, and column chemistry affect separations in UPLC by combining improved efficiency with manipulations of selectivity and retentivity to develop high-resolution chromatographic methods.

The automation of this approach is implemented with a new, low-dispersion, 4-position column selection device which has been introduced to effectively and efficiently screen multiple variables.

In this lecture, we discuss the chromatographic effect of chemical factors on retentivity and selectivity. By understanding these effects, we can formulate a systematic screening protocol that streamlines the methods development process.

A SYSTEMATIC APPROACH TO CONVERT UPLC CONDITIONS TO HPLC CONDITIONS

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UPLC has gained popularity since its introduction in 2004. UPLC provides faster analysis time and higher efficiency compared to regular HPLC. However, a method that is truly optimized for UPLC cannot be run directly on a traditional HPLC system, mainly due to pressure limitations. It can be a dilemma for analysts or lab managers when deciding which technique will be used for a routine analytical method since they are considered two different methods and there is no easy way to transfer (or translate) methods between UPLC and HPLC to ensure, most importantly, equivalent selectivity.

A systematic approach to convert UPLC conditions to traditional HPLC conditions will be discussed. In this approach, DryLab software was used to facilitate translation of UPLC conditions to traditional HPLC conditions. A combination of four temperature and gradient scout runs was performed under UPLC conditions. The retention times and peak areas of each component for each experimental run were entered into the DryLab software to establish a retention model for

UPLC conditions. This DryLab retention model for UPLC was verified by actual verification runs to compare to those predicated by DryLab. Next, the column parameters dwell volume and flow rate were changed from UPLC parameters to traditional HPLC conditions within the DryLab software to predict separation performance.

In summary, our experiments showed that (1) DryLab can be used to accurately optimize UPLC separations and (2) can accurately translate UPLC conditions to traditional HPLC conditions and predict retention behavior very well (provided that the selectivity of UPLC and HPLC columns is the same).

COMBINATION OF NEWLY DEVELOPED TECHNOLOGY AND COMMERCIAL DETECTION EQUIPMENT FOR SIMULTANEOUS RAPID ACCURATE DETECTION OF CHEMICAL WARFARE AGENTS AND BIOLOGICAL TOXINS

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In crisis and consequence management against biological and chemical terrorism, monitoring, detection, and identification are important for prevention of terrorism occurrence and minimization of damage. Among terroristic weapons, chemical warfare agents (CWAs) and biological toxins (toxins) show toxic effect within one day, and so on-site detection is desirable. We have evaluated commercially available on-site detection equipment for CWAs and toxins, and elucidated shortcomings of the present on-site detection system conducted by first responders: low sensitivity against gaseous CWAs and toxins, high false positive frequency, and tedious operation. To overcome the difficulty in reliable on-site detection activity, we have developed three new technologies: monitoring tape method (MTM), counterflow introduction atmospheric pressure chemical ionization mass spectrometry (CFI-APCI-MS), and sugar-based chip biosensor method (biosensor). MTM enables sensitive (LOD: sub mg/m³) and accurate detection of blood and choking agents within 1 minute, using portable multiarray diffusion-type apparatus. CFI-APCI-MS enables very sensitive (LOD: sub μg/m³) and continuous monitoring of volatile (nerve gas and blister agent) and nonvolatile (vomit agent and tear gas) CWAs, using ion-trap type analyzer. Biosensor enables sensitive (sub n/mL level) detection of ricin and botulinum toxins, using specific carbohydrate ligands and surface plasmon resonance measurement. By combining these newly developed technologies and commercially available equipment such as ion mobility spectrometer, it is possible to detect thorough CWAs and toxins sensitively, rapidly, and accurately without tedious operation.

A NOVEL CHIP-BASED SENSING SYSTEM DEVELOPED FOR HAND-HELD AND ONLINE SPECTRAL MEASUREMENTS

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The concept of a “spectrometer on a chip” has been discussed now for many years. Although certain specialized measurement systems have been developed featuring customized electronics, there has not been any form of general-purpose instrumentation based on a chip-based optical spectrometer.

A miniaturized spectral sensing system has been developed based on an optical chip assembly measuring approximately 8 mm × 1 mm. The sensor, based on a 128-element optical array, is capable of providing spectral data from the UV to the near infrared. The device has been integrated into a spectral measurement engine that includes solid state light sources, on-board signal acquisition, data conditioning, and spectral data extraction. The entire package has been designed to be accommodated on a single board system with accompanying electronics for information display and communications. This compact system does feature conventional optics and is designed to be constructed from inexpensive molded parts. The result is an optimized, versatile sensing system that can be easily integrated into different measurement platforms.

This paper will discuss the spectral sensing concept and its application to spectral absorption and fluorescence-based measurements. In its current manifestations, the spectral engine is applied to hand-held and online systems that feature integrated sample handling. The on-board intelligence provides the storage of a large number of methods that can be applied to a host of industrial, environmental, and biological applications. Examples presented will include both water-based and organic chemistries.

FROM LABORATORY TO PRODUCTION IMPLEMENTATION ON NEAR INFRARED SPECTROSCOPY FOR ONLINE DETERMINATION OF MOISTURE IN FLUID BED DRYERS

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It has been demonstrated that near infrared spectroscopy (NIRS) is an ideal technology for moisture determination in fluid bed dryers for the pharmaceutical industry. There are numerous publications to support this. However, the actual implementation in a production environment, to the best of our knowledge, has not been demonstrated. This paper describes in detail the several stages required for a successful installation, from feasibility testing in a laboratory environment through to the validation of the entire process.

DEVELOPING ROBUST CHEMOMETRIC TOOLS FOR ONLINE SPECTROSCOPIC DETERMINATION OF "PHARMACEUTICS": A CALIBRATION TRANSFER AND QUALITY OF DESIGN STUDY

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Over the years, near infrared (NIR) spectroscopy has been coupled with multivariate techniques to determine concentration of active ingredients and excipients in pharmaceutical drug products. Typically, quantitative aspect of chemometrics analysis involves the generation of multivariate calibration models from a set of data collected on a particular NIR instrument.

When implementing chemometrics-based algorithms for online spectroscopic systems, it is critical that the dataset of the calibration model be representative exhibiting the full range of variations expected for the eventual unknown sample. The variations could be any spectroscopically sensitive parameters, such as instrumental differences, instrumental drift, and chemical or physical properties of drug substance and excipients. However, due to the difficulty, if not impossibility, in incorporating foreseeable variations into the original calibration model, solely relying on simple chemometrics-based algorithms is risky. It is essential for the pharmaceutical industry to adopt the development of specialized calibration transfer (SCT) algorithms as well as using these algorithms to evaluate any spectral variances in the data.

In this research, various techniques will be evaluated on their efficiency and effectiveness in handling the problems encountered during calibration transfer. The data analysis will be based on datasets collected on multiple NIR instruments over a period of weeks. Spectral variances will be investigated mainly based on measurement of solid dosage from samples.

MULTIVARIATE PARAMETER RANGES MAXIMIZATION FOR TABLET PROPERTY OPTIMIZATION IN THE DEVELOPMENT OF A PHARMACEUTICAL FORMULATION

Husheng Yang

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A pharmaceutical tablet usually carries several desired properties to facilitate the delivery of the active pharmaceutical ingredient (API). Since many tablet properties are often affected by several parameters in raw materials and processes, pharmaceutical companies have started using multivariate analysis increasingly to achieve desired properties in the development of a tablet formulation. To accommodate the most variability and flexibility in a formulation, it

is often needed to maximize the ranges of several parameters that affect a tablet property. The aim of this work is to find out an accurate and effective way of maximizing several parameter ranges simultaneously for a tablet property development.

In this work, an accurate multivariate model was first established to correlate a tablet property with all the parameters that could affect the property. Three methods were then used to maximize the ranges of all the effective parameters so that the desired tablet property could always be achieved within the calculated ranges. The first method used Monte Carlo simulation to obtain a large amount of synthetic data for the extraction of parameter ranges. The second method used a function optimization algorithm with the multivariate model as the objective function to obtain the parameter ranges. The last method used response surface methodology with polynomial models to obtain the parameter ranges. The results of these three methods were compared for accuracy and ease of use.

AUTOMATED PH MEASUREMENTS OF PURGE AND TRAP WATER SAMPLES FOR USEPA VOC METHODS

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Many USEPA methods require that all drinking water and waste water samples be preserved with acid during the collection process to pH less than 2 prior to analysis for volatile organic compounds (VOCs) by purge and trap (P&T). Water sample preservation prevents degradation prior to analysis, and each analytical method describes a unique preservation technique. Some of the methods requiring acid preservation prior to analysis are USEPA methods 502, 524, 624, 5030, and 8260. Other regulatory VOC methods, such as the Massachusetts volatile petroleum hydrocarbon (VPH) method, offer the option of preserving the sample by raising the pH of the sample above 11.

For nearly thirty years, the process of measuring, recording, and reporting each sample's pH level has been an entirely manual procedure. The technician or chemist must open each sample vial, measure the pH manually using either pH indicator strips or a pH probe, record the measurement in a logbook, and then include the measurements in the final client report. For high-throughput laboratories with multiple P&T instruments analyzing samples 24 hours each day, this practice can be extremely time-consuming, labor-intensive, and expensive.

This poster describes a new instrument that fully automates the entire process of measuring, recording, and reporting the pH level for all VOC water samples to verify that the pH level meets the requirements specified in regulatory methods. The new instrument improves laboratory productivity and profitability by eliminating the labor and materials required for manual measurement of pH levels of VOC water samples.

REAL-TIME MONITORING OF POLYMER HEAT CURING BY DIAMOND ATR SPECTROSCOPY

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Epoxyes were first introduced commercially in the early 1950's for the aerospace industry and are now widely used for a variety of applications, including manufacturing of commercial vehicles, electronics, inks, and varnishes. Epoxyes consist of two components: a resin and a curing agent or hardener, which are tailored for specific properties such as adhesion, elasticity, and hardness. For the best performance, the two components are mixed and cured under controlled conditions.

It is important to have real-time methods of analyzing the epoxy curing process to determine the optimum curing conditions for a given epoxy, for quality-control purposes, and to better understand the kinetics and underlying chemical processes. Spectroscopic methods for examining epoxyes curing in real time are not common since many epoxyes are not readily removed from the windows or crystals used for transmission or ATR measurements.

The introduction of the diamond ATR crystal now makes real-time monitoring of epoxyes curing feasible. Diamond is extremely hard so the bonded epoxy can simply be scrapped off. This paper examines the curing of an epoxy under controlled elevated temperatures in real time using a new heated diamond ATR.

A LOW-COST AUTOMATIC GRAVIMETRIC TITRATION SETUP FOR COLLEGE LABORATORY

Alexander Y. Nazarenko

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With widespread availability of electronic balances, a novel simple approach to gravimetric titrations becomes an attractive alternative to usual syringe-based automatic titrators. The tested setup consists of electronic balances with RS-232 or USB connection to the PC, a vessel for titrant solution, delivery system, and an appropriate detector that is also connected to the same PC via suitable data logging device. A number of detectors for various types of titration were tested: a pH electrode, a pAg electrode, Pt electrode for redox titrations, and a fiber optics probe connected to the CCD-based spectrophotometer. Simultaneous collection of the mass of titrant vessels and corresponding detector readings produces satisfactory titration curves suitable for automatic titration with three-digit precision.

Several variants of delivery system are discussed. Modifications of the usual titration procedures are described.

The suggested experimental setup presents a low-cost option for automatic titration in a college laboratory.

ONLINE HPLC+HRGC COUPLING: A NEW, FULLY AUTOMATED METHOD FOR THE DETERMINATION OF PESTICIDES IN VEGETABLE SAMPLES

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Determination of organic contaminants is regulated by governments all over the world and has been included as priority pollutants in the European Union lists. Pesticides constitute a very important group of these compounds owing to their high toxicity and their extended use in agricultural practice.

The standard method for the analysis of pesticides in vegetable samples is chromatography with selective detectors and eventually GC-MS. Due to the complex matrices, complicated sample cleaning procedures are needed, previously to the HRGC analysis. Classical methods consist in a pesticide extraction with ethyl acetate from the vegetable; then the supernatant is evaporated to dryness and finally dissolved to obtain the solution ready to inject into the GC.

In this work, a new application of the patented TOTAD interface for online coupling HPLC-HRGC is presented. The interface coupling an HPLC to an HRGC in the KONIK K2 HPLC+HRGC system allows the direct analysis of pesticides in fruits and vegetables.

The pesticides are extracted from the sample, previously ground, by a simple stirring and with a few milliliters of organic solvent placed directly in the injection vial. After the extraction, the supernatant is injected directly into the HPLC system and the fraction of interest is transferred to the HRGC by using the interface. With the addition of the KONIK Robokrom HPLC autosampler and the full control through the Konikrom software, the complete analysis can be easily automated and performed in a few minutes, limiting the use of solvents while protecting sample integrity.

The extraction step and the HPLC and HRGC parameters were optimized to obtain a multiresidue method with the selectivity and sensitivity required for the legislation. Finally, the method was applied to real samples and results compared in terms of selectivity and sensitivity with the standard methods of extraction.

NEW MASS SPECTROMETRIC SYSTEM FOR COMBUSTION ENGINE DEVELOPMENT: ULTRAFAST IN-CYLINDER MEASUREMENT OF OIL EVAPORATION

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Car emission standards are getting more stringent in the future.

To reduce the emission from combustion engines, the highly dynamic process has to be investigated in order to understand the generation of pollutants better. This means that

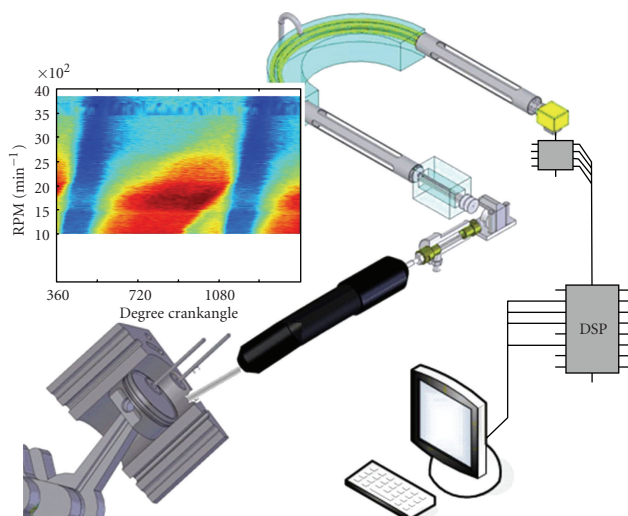


FIGURE 1

the gas composition inside the cylinder has to be monitored online for each combustion cycle.

Part of the online measuring system under development is the Lubrisense 1200 mass spectrometer equipped with a CFD optimized high-temperature direct inlet. The inlet-system consists of a transfer capillary, two differential-pressure stages, a heating system, and a pressure control device. Together with a high-speed data acquisition system (12.5 kHz), this system enables crank-angle-resolved monitoring of oil evaporation in the combustion chamber over the full speed torque range of an engine. Rapid online measurements will yield further information to understand the process generating pollutants.

DEVELOPMENT AND OPTIMIZATION OF A GLUCOSE OXIDASE-MODIFIED ROTATING DISK ELECTRODE

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Enzyme electrodes combine the high sensitivity of electrochemical detection with specificity of the enzyme for its substrate. Enzyme electrodes have gained popularity in analysis of clinical, environmental, and food samples due to being relatively simple, inexpensive, and easy to prepare. Enzyme electrodes also provide rapid analysis, easily regenerate, and are reusable. Adding a rotation to the electrode provides continuous stirring of the bulk solution and faster transport of the analyte to the electrode surface resulting in shorter detection times and faster overall analysis. However, rotating disk enzyme electrodes (RDEEs) are less commonly studied and not commercially produced. Rotating disk electrodes (RDEs) modified with glucose oxidase (GOx) were constructed as working electrodes for the amperometric detection of \bullet -D-glucose. The rapid rotation causes the enzyme to leach off

over time; a dialysis membrane was added to trap the enzyme on the electrode. The enzyme, glucose oxidase (GOx), which is highly specific for \bullet -D-glucose, was directly immobilized onto the Pt electrode surface through two different immobilization methods. An electrostatic/surface adsorption and a polymer entrapment method were both used to immobilize the enzyme to the Pt RDE electrode. Glucose was detected at 650 mV versus Ag wire pseudo-reference electrode using a three-electrode configuration including a Pt wire as the auxiliary electrode. Experimental conditions such as detection potential, pH, temperature, and electrode rotation rate were optimized for the electrostatic/surface absorption method. The surface area of the electrode was determined using chronocoulometry. The specificity of the GOx RDEE for \bullet -D-glucose relative to sweeteners structurally close to \bullet -D-glucose was also studied.

ACKNOWLEDGMENT

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EVALUATION OF REAL-TIME IMMUNO-PCR ASSEMBLAGES FOR THE FORENSIC IDENTIFICATION OF SEMEN STAINS

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The identification of a biological fluid stain at a crime scene as a semen stain is often critical to the assessment of the crime. This is particularly true in date rape cases, where the victim was drugged or incapacitated and have no direct memory of a sexual assault. If there is no sperm in a sample, identification of a biological fluid stain as semen is difficult. All semen stains, even those devoid of sperm, will contain the protein prostate specific antigen, or PSA. PSA is at its highest level in semen compared to other body fluids. This investigation combines the use of the chemical specificity associated with immunotechniques with outstanding sensitivity attained in real-time PCR. Antibodies specific for PSA are immobilized to surface of a vessel. When sample is introduced, PSA interacting with the bound antibody is then immobilized. A second detection antibody with a coupled DNA molecule is added which binds to the to the immobilized protein target. The sample vessel is then washed to free all unbound reagents and the DNA is amplified and detected with a fluorescent tag. The real-time PCR was performed to amplify and detect fluorescently tagged DNA. There is no need to quantitate the DNA; simply the presence of the previously immobilized DNA indicated the presence of PSA. Using three different assemblage strategies provided by TATAA Biocenter, this study compares the efficacy of these assemblages in detecting PSA in semen samples devoid of sperm.

THE DETECTION OF SUGARS BY ELECTROGENERATED CHEMILUMINESCENCE WITH RUTHENIUM(II) TRIS-BIPYRIDYL AT GOLD AND GLASSY CARBON ELECTRODES IN A FLOW-STOP THIN LAYER CELL SYSTEM

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The electrochemiluminescence (ECL) of Ru(bpy)₃²⁺ was studied in the presence of sugars at gold (Au) and glassy carbon (GC) working electrodes having a surface area of 7.06 mm². In an oxidative-reduction mechanism similar to that of Ru(bpy)₃²⁺-Tripropylamine (TPA), a sugar reacts with Ru(bpy)₃²⁺ whose excited state (Ru(bpy)₃^{2+*}) emits light at 610 nm. Using cyclic voltammetry in a flow-stop thin layer cell analysis system, an ECL signal is observed from anolyte solution containing Ru(bpy)₃²⁺, Triton-X, buffer, and a sugar. At both Au and GC electrodes, a linear ECL response is observed with increasing sugar concentration. A limit of detection (LOD) of approximately 1 mM was found for all sugars studied. Solutions were prepared in a 1 M phosphate buffer pH 11, 1.0 × 10⁻⁸ M Ru(bpy)₃²⁺, and 0.01/0.02% (v/v) TX-100.

DETERMINATION OF MERCURY SPECIES IN PORTUGUESE SALT MARSHES USING CAPILLARY GC: ATOMIC FLUORESCENCE SPECTROMETRY

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Salt marshes located near to industrialized areas can act as natural sinks for trace metals. Anthropogenic metals, associated with suspended particulate matter, can be transported by tidal currents and trapped by vegetation with subsequent incorporation into sediments. Plant roots can interact with the surrounding sediment, exuding oxygen, and organic compounds that influence the distribution and availability of trace metals; however, the amount of metals taken up by the plants is dependent on the metal availability in the sediment, and this is modified by the root activity. Oxygenation of upper estuarine sediments decreases rapidly with depth due to the consumption of oxygen in the oxidation process of the organic matter; however, salt marsh sediments receive an additional input of oxygen in the subsurface layers through the well-developed aerenchyma of salt marsh plants (halophytes) which transports oxygen from leaves to roots and consequently to the surrounding sediments. This supply of oxygen can alter significantly the redox status of sediments with strong repercussions on the biogeochemistry of nutrients and trace elements, namely, mercury. The methylation of mercury in salt marsh sediments is poorly documented, although abundant microorganisms and strong redox gradients between roots and surrounding sediments may

favor that process. Because the sediment environment in salt marshes is exceedingly complicated, it is pertinent to investigate the conversion of inorganic mercury into organomercury species. A fully automated GC coupled to atomic fluorescence spectrometer will be described for the determination of methylmercury in sediments and salt marsh plants.

NOVEL APPROACH IN THE PROFILING OF VOLATILE ORGANIC COMPOUNDS IN WATER UTILIZING THE PURGE AND TRAP AUTOSAMPLER

Teri Dattilio and Ed Price

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EPA methods 524.2 and 624 outline the general criteria for volatile organic compounds in water and wastewater analysis. In recent years, laboratories have run into an instrumental barrier in their efforts to increase throughput and productivity for the analysis of Volatile Organic Compounds (VOCs). Conventional purge and trap technology had reached a limit where the speed could not be increased further without severely affecting analytical performance and quality. This paper compares the standard purge and trap technology and the new tekmar purge and trap showing that reduced run time can be achieved while maintaining EPA compliance with improved data quality.

NEW APPROACH IN THE PROFILING OF VOLATILE ORGANIC COMPOUNDS IN SOIL UTILIZING THE PURGE AND TRAP AUTOSAMPLER

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EPA methods for the determination of volatile organic compounds in soil following method 5030 and 8260 parameters can be a challenge when trying to ensure the fastest possible run time without sacrificing your analytical integrity. Two main aspects that are often compromised when optimizing a purge and trap concentrator and autosampler are carryover and low-end sensitivity. Teledyne Tekmar is offering a new approach to purge and trap optimization that is achieving unprecedented performance. Data presented demonstrate advances in analytical abilities without sacrificing any data integrity.

AUTOMATED DISSOLUTION/REACTION MONITORING IN A VESSEL SAMPLED BY A NEW "SIPPING" AUTO SAMPLER

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A fast method for real-time reaction monitoring is demonstrated using capillary chromatography and a new automated

reaction monitoring tool—"the sipper." Aliquots are taken automatically from the reaction vessel using a fully programmable method. After online dilution the "sipper" transfers the sample to a capillary HPLC system where fast chromatography is performed. Monitoring reactions in this way allows a full time course of the reaction to be plotted so that conditions may be optimized.

INTELLIGENT SOFTWARE SOLUTIONS FOR COMPLEX IC SYSTEMS

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It is only recently that software tools for liquid chromatography have gained a foothold in the HPLC/IC field. Several software programs compete for the market and appeal to the user community. Thus, today's situation is similar to that of office software tools several years ago.

In the latter field, a tremendous concentration process has left over only very few products that were able to resist the tough competition and persisted on the market. As a result, these software tools have become standards and everyday tools for an overwhelming majority of users. On the other hand, users see their choices drastically reduced.

Given that background, are we heading towards a similar, quasimonopolized software situation in the field of IC? Certainly customers wish to select among different software solutions the one whose features correspond best to their applications.

It might be the actual spreading of a given product and good marketing pushing a certain software environment that mainly decide about winning or losing customers. Although highly important, these points will not be treated in our presentation.

We concentrate on features that future IC software must provide in order to keep its place within the shrinking group of high-end products. Accordingly, we highlight decisive software characteristics that are crucial for performance as well as for user acceptance. Examples are as follows:

- (i) excellent visualization of configuration, current determination, method, instrument control, and databases;
- (ii) intelligent recognition of instruments and accessories;
- (iii) control of a practically unlimited number of devices by mouse click;
- (iv) management of all devices with one time program, including parallel tasks, sample preparation, sample processing, and so forth;
- (v) efficient algorithms for data evaluation and databases;
- (vi) user-based client-server systems;
- (vii) GLP and monitoring features;
- (viii) potential for growth regarding the capabilities of the instruments, the laboratory, the methods in use, and the data already recorded;
- (ix) flexibility ranging from "one button IC" for the unskilled user up to the administrator level;

- (x) standardized interfaces: AIA import/export;
- (xi) straightforward, self-explaining dialogs for complex instruments and tasks.

We expect that software products will further contribute to a healthy variety of solutions if they succeed in managing the complexity of modern ion chromatography in an intelligent way.

COMPUTER ASSISTED CALCULATION OF OPTIMAL STATIONARY PHASE COMPOSITION FOR HPLC METHOD DEVELOPMENT

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Phase optimized liquid chromatography (POPLC) is a method to find out the optimal stationary phase composition for each particular separation. Due to the fact that retention times are additive, different stationary phase segments are coupled together to create the column with the best overall performance for each particular separation. The retention time for each analyte is controlled by the use of the best selective column for this purpose. This column is calculated by computer software. For the calculation of the optimal column composition, the measuring of the retention data for each of the compounds of the mixture on each of the different stationary phases is required.

In this paper, we demonstrate the optimization of a separation by coupling different stationary phases of different brands. It can be shown that an extreme improvement in selectivity can be achieved just by coupling existing columns from the shelf. An easy procedure of how to apply the software in daily method development will be presented.

AUTOMATED HIGH-THROUGHPUT FORMULATIONS DEVELOPMENT

Cliff Olson and Werner Zinsser

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The continuous rise in costs for pharmaceutical products forces us to take a closer look at traditional workflows. So far in the early stages of drug discovery, the focus was on the chemical effects, leaving the analysis of the physical effects using formulation and solubility studies as the last link in the process.

This approach could lead to the terrible consequence of missing important required properties for dosage, solubility, storage, and so forth. All the money spent on the discovery so far would have been totally wasted.

To avoid more disappointment and financial disaster in the final development stage, it is crucial to have already eliminated unsuitable compounds at an earlier stage. New workflows and hardware systems are required which enable us to deal with the small volumes of material available at the early discovery stage in order to study the physical and chemical properties.

Zinsser Analytic has developed an automatic workbench which enables you to execute a workflow to test formulation conditions in parallel. The reactor is based on the microplate format for 8 to 24 vials with different compounds or different testing parameters. Tools for pH measurement and adjustment, precision dosage, weighing, and mixing (even of highly viscous media) are provided. Optical control, evaporation, filtration, and HPLC/LC-MS integration can be provided enabling you to carry out your well-proven formulation methods and solubility tests on a single platform.

HIGH-THROUGHPUT BLENDING FOR AUTOMATION OF LUBRICANT FORMULATIONS

Cliff Olson and Werner Zinsser

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The blending station from Zinsser Analytic is a new automated system designed for the preparation of complex blends of viscous media, ideal for developing high-throughput liquid formulation testing experiments. Specially designed pumps, liquid delivery tools, and powerful software allow high-throughput blending without compromise in precision or accuracy.

The blending station is based on a liquid handling workbench with two independently operating arms. Each arm is equipped with the viscous media dispenser and a gripper. Gripping tools are designed for transportation of the blending vessels and the tips of the viscous media tools. Bulk reagents in 5-liter cans are stored under the workbench or outside the cabinet. A pump tower under the platform with 9-high precision rotating piston pumps delivers the reagents from the reservoirs and dispenses them via a special dispensing head into the blending vessels waiting on the two balances. These two precision 4-place weighing cells are integrated in the middle of the deck of the workbench. The blending vessels are transported by the robotic arms from the storage racks to the balances, tare weighed, filled with the viscous liquid, and then brought back to the storage rack.

Standard low-cost glass jars are used as blending vessels (60 mL, 150 mL, 200 mL, and 300 mL). Three individually controlled heated zones on the deck (80°C, 50°C, and room temperature) contain racks with the blending vessels, source containers for low-volume materials, and the dispensing cartridges of the viscous dispensing tool.

ONLINE PROCESS TITRATOR SYSTEM

Tore Fossum

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A repetitive sampling online titration system (Figure 2) was configured using a laboratory benchtop titrator by adding pumps, a sampling valve, and an analog output to communicate with a PLC process controller. All of the components are available off the shelf. The system has provision for automati-

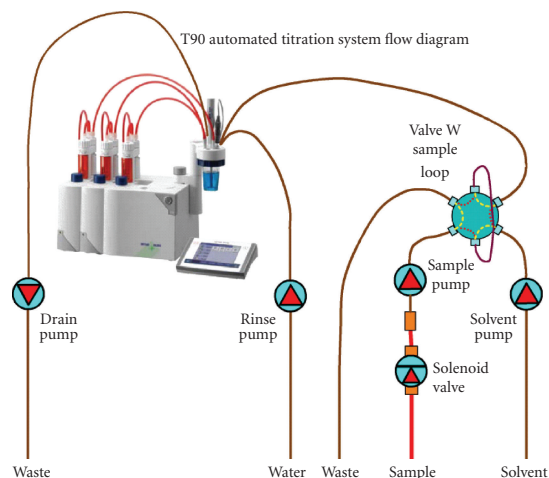


FIGURE 2

cally calibrating the electrode and for running check samples. Precision for determining acidity was better than 0.5% rsd.

AUTOMATION OF H5N1 DETECTION KIT WITH VERSA MINI PCR WORKSTATION

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In the recent years, influenza virus H5N1 is being closely monitored by public health authorities for being a health problem. For molecular detection of this virus, automation is also becoming an important tool. At Aurora Biomed Inc., automated sample preparation for the detection of viral RNA from influenza A/H5N1 was developed with a commercially available kit. Liquid handling for various components of the kit was carried by the workstation for 50 μL assays in sterile 500 μL reaction tubes. The reaction tubes were subsequently taken to the thermocycler where reverse transcription and amplification took place in the same buffer system.

The amplified products of about 190, 190, 107 bp on 3% agarose gel indicated that the workstation can be used for the kit. RNA being very sensitive to degradation and PCR being a sensitive process to volume, concentration of different assay components, and contamination issues, the results showed that the workstation conformed to the precautionary requirements of the kit.

HIGH-THROUGHPUT ADMET PROFILING EMPLOYING A NOVEL LOW-VOLUME PARALLEL LIQUID CHROMATOGRAPHY SYSTEM

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Analytical chemistry constitutes a significant component of the drug discovery and development process. Many of the traditional techniques employed for the evaluations of

compound physicochemical properties are often inadequate in modern HTS due to the throughput and sample amount limitations imposed by these approaches. Other approaches can provide adequate throughput, but they are not sensitive to compound purity and identity. Micro parallel liquid chromatography (μ PLC) offers the advantages of a separation-based approach (such as HPLC and LC-MS) and enables high-throughput chemical analysis for the characterization of ADMET parameters and physicochemical properties. The approach has also been shown to reduce sample consumption, solvent usage, and waste generation.

The μ PLC system employed for the determinations highlighted in this presentation is equipped with 24 parallel columns for liquid chromatography, each with its own sample introduction port and exit port for connection to detector(s) of choice. Flow from a binary solvent delivery system is divided evenly across 24 channels and results in 1/24th of the programmed pump flow rate through each column. Samples are introduced to the columns (housed in a cartridge) by a multichannel autosampler, which is configured to sample from either 96-well or 384-well SBS standard plates.

In this presentation, results from the μ PLC characterization of compound lipophilicity (based on logP values), solubility (thermodynamic and kinetic), purity, and stability will be discussed and compared with those obtained with conventional HPLC instrumentation and with other commonly used techniques (such as nephelometry, flow cytometry, and UV plate readers for the evaluation of compound solubility).

HIGH-THROUGHPUT ANALYSIS BY DESORPTION ELECTROSPRAY IONIZATION

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A recent innovation in mass spectrometry is the ability to record mass spectra on ordinary samples, in their native environment, without sample preparation or prepreparation by creating ions outside the instrument. In desorption electrospray ionization (DESI), electrically charged droplets are directed at the ambient object of interest; they release ions from the surface, which are then transferred through the air into a conventional mass spectrometer. Extremely rapid analysis is coupled with high sensitivity and high chemical specificity; even complex matrices such as blood, serum, urine, and tissue can be analyzed directly. These characteristics are advantageously applied to high-throughput metabolomics, natural products discovery, pharmaceutical analysis, and explosives detection among other applications. DESI is applied to the rapid direct analysis of plant tissues; all the previously reported alkaloids have been detected in *C. maculatum* along with fifteen out of nineteen known alkaloids for *Datura stramonium*. Positive and negative ion DESIs are used to characterize pharmaceutical samples formulated as tablets, ointments, and liquids at speeds of up to 3 samples per second. For metabolomics applications, DESI re-

sults are combined with NMR and advanced statistical tools to differentiate lung cancer from control samples by studying the metabolite content of mouse urine. Inborn errors of metabolism are being studied by this method. Breast cancer (profiling) and prostate cancer (hormone response study) serum samples are being investigated to differentiate the different cancer types and study disease progression. DESI can be used as a high-throughput screening or diagnostic tool in targeted metabolomics applications. Future possible uses of DESI could involve in vivo clinical analysis, early diagnosis, and adaptation to miniature mass spectrometers.

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NEW APPROACHES TO RAPID PHARMACOKINETIC AND METABOLIC SCREENING IN EARLY DRUG DISCOVERY USING UPLC-MS/MS

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There is an increasing demand, within the pharmaceutical industry, for higher-throughput pharmacokinetic and metabolic screening. The rationale behind this undertaking lies in the advancement of lead compounds that have the highest probability for success. We have approached this task from a drug metabolism/pharmacokinetics perspective with Liquid Chromatography Mass Spectrometry/Mass Spectrometry (LC-MS/MS) as our principal tool in an effort to satisfy these demands.

We have implemented a novel pharmacokinetic paradigm called cassette-accelerated rapid rat screening (CARRS) and a similar assay for monkeys and dogs. This rugged assay comprises the use of fast LC-MS/MS and highly organized interdepartmental collaboration between chemistry, animal dosing facility, and drug metabolism to screen 60 new compounds per week. In order to achieve rapid turnaround time, high quality, and informative data, we have utilized a variety of approaches that will be discussed in this paper.

Our most recent approach utilizes ultra performance liquid chromatography (UPLC) tandem mass spectrometry. UPLC provides improved chromatographic separation and sensitivity while diminishing carryover. We have used the waters acuity UPLC in conjunction with both the Micromass Premier and TSQ Quantum triple quadrupole mass spectrometers. We have developed a generic approach to rapidly provide pharmacokinetic information by using automated method development software such as QuanOptimize and QuickQuan. This EZ-CARRS approach has facilitated significantly improved turnaround time including same day reporting of data. Additionally, we have introduced a metabolic profiling assay (in plasma) that is connected to the CARRS assay, allowing for the routine profiling of both common and unique metabolites including estimated AUCs without an authentic standard.

These approaches have had a major impact within drug discovery in giving program teams the ability to make decisions on the fly.

HIGH-THROUGHPUT SIMULTANEOUS DETECTION AND IDENTIFICATION OF REACTIVE INTERMEDIATES USING NEGATIVE PRECURSOR ION SCANS COMBINED WITH POSITIVE PRODUCT ION SCANS

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Reactive drug intermediates can cause hepatotoxicity. It can be caused through covalent modification of key proteins. To predict the potential of in vivo reactive intermediate formation, we have developed an in vitro assay in which compounds are incubated with microsomes fortified with glutathione (GSH) and cofactor NADPH. The resulting GSH conjugates were commonly detected using positive electrospray and neutral loss (129 Da) of pyroglutamic acid from the $[M + H]^+$ ion of the GSH conjugates. However, not all classes of GSH adduct afforded this neutral loss upon collision-induced dissociation. In this study, 12 commercially available compounds were incubated in human liver microsomes. Samples were run using constant neutral loss (CNL) of 129 in positive mode, and precursor ion scan of 272 in negative mode ($-Prec$) followed by enhancing resolution ($+ER$) to switch from negative to positive mode, and information dependence acquisition (IDA) of the detected GSH conjugates using enhanced product ion scan ($+EPI$) on the API 4000 Q-Trap equipped with turbo ion spray source. When CNL was used, GSH adducts of 10 out of 12 compounds could be detected. In contrast, GSH adducts of all 12 compounds were readily detected in human liver microsomes using $-Prec$. Although all the GSH adducts were detected using $-Prec$ of 272 Da, the relative intensities varied. These might be dependent on the stability of the adducts, the amount formed, and collision energy used. Enhanced product ion spectra were also acquired for identification and confirmation simultaneously. In conclusion, $-Prec$ of the 272 fragments, followed by ER and $IDA EPI$, provides a sensitive and specific analytical technique that can be applied for detecting, structurally identifying, and confirming GSH conjugates of various unknown compounds.

CHEMICAL FINGERPRINTING OF DIESEL FUELS USING GC-MS AND CHEMOMETRIC METHODS FOR FORENSIC ANALYSIS

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Characterization of petroleum fuels is of importance in forensic arson investigations. Comparison of a fuel sample recovered from a crime scene to one in a suspect's possession

is difficult due to the complex chemical nature of the fuel. Currently, gas chromatography mass spectrometry (GC-MS) is a valuable technique for analyzing petroleum samples, such as diesel. Chemometric methods can be used to statistically classify or group fuel samples based on the chemical fingerprint from the GC-MS.

Twenty-five diesel samples were collected from the Lansing, Michigan area and analyzed by GC-MS. Sample components were identified from the mass spectra by comparison with a spectral database. Mass-to-charge ratios were identified to represent aliphatic (m/z 57) and aromatic compounds (m/z 91). The diesel samples were evaluated using both the total ions (TICs) and extracted ion chromatograms (EICs) for the representative m/z values. The chromatograms were retention times aligned, normalized, and mean-centered prior to chemometric analysis.

Pearson correlation measures the strength of the association between two chromatograms, where a correlation coefficient near unity indicates that the diesel samples are similar in origin. Correlation coefficients for the TICs ranged from 0.889 to 0.996 for samples of known similar origin, while those of dissimilar origin were as low as 0.753. Correlation of the EICs provided greater discrimination of diesels, with samples of known similar origin ranging from 0.966 to 0.994 and 0.968 to 0.995, while those of dissimilar origin were as low as 0.888 and 0.652 for m/z 57 and m/z 91, respectively.

Principal component analysis (PCA) is a multivariate technique that reduces the dimensionality of the dataset, while retaining the important characteristics of the original data. PCA of the TICs suggests the formation of two separate clusters with two isolated outlying samples. While similar clustering is observed for each of the EICs, greater variance is observed within each cluster. The distinct clustering suggests chemical similarity, as one cluster consistently contains only one brand of diesel. Taken together, these methods show promise for the association of forensic fuel samples of similar origin and the discrimination of samples of different origin.

THE IDENTIFICATION OF NEW NOVEL BIOMARKERS OF DISEASE AND TOXICITY USING UPLC/MSE AND ADVANCED MULTIDIMENSIONAL STATISTICAL ANALYSIS

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One of the major scientific challenges of the 21st century will be determining the relationship between the human genome and the risks of developing major diseases such as cancer, diabetes, arthritis, and so forth. Understanding the relationship between the genome, the proteome, and the expressed endogenous metabolites (metabolic phenotyping or metabotyping) requires the development of ever more powerful analytical chemistry techniques and data interpretation tools. In the metabonomics arena, this task is normally carried out by proton NMR, GC/MS, or LC/MS, the latter with exact mass analysis, and multivariate statistical analysis. Interesting

ions in the LC/MS data highlighted by the multivariate analysis are subsequently reanalyzed by using LC/MS/MS for structural ID. Traditionally, mass spectrometers lacked the MS/MS duty cycle required to collect product ion scan information for thousands of metabolite ions simultaneously. In this paper, however, we will describe how the application of ultra performance LC with the simultaneous acquisition of both precursor and product ion MS data has been combined with novel statistical heterospectroscopy, and applied to this task. Urine samples were collected from two studies: (1) male control and male diabetic rates over a 16-week time period, (2) male rates following the administration of Hydrazine, at 1 and 80 mg/kg. The samples were analyzed by UPLC/MS using the simultaneous acquisition of alternating low and high collision cell energy, resulting in significant increase in MS duty cycle followed by this new form of statistic on the resulting data. The reversed-phase UPLC/MS analysis resulted in chromatographic peak widths in the order of 2 seconds at the base. This new approach revealed extra information about the dataset and allowed the easy identification of the precursor and product ions. It was possible using mass deficiency filters to link these ions to obtain MS/MS structural information.

DEVELOPMENT OF A MICROFABRICATED ELECTROCHEMICAL DETECTION SYSTEM

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Microscale separations such as capillary liquid chromatography (LC) and capillary electrophoresis (CE) offer shorter analysis times, low reagent and solvent consumption, increased reliability, and high performance over traditional separations. The use of microfluidic devices to perform these types of separations provides advantages in instrumental integration and portability. The increasing popularity of capillary LC and CE over the last 25 years and the more recent transition to microfluidic devices in the last 15 years have created a need for detection systems that are amenable to miniaturization. Due to the low flow rates and very small volumes used in capillary LC and CE, these systems must provide very high mass sensitivity and chemical selectivity and have the ability to measure analytes of interest in intended applications without prior chemical derivatization. Additionally, detectors should be easy to use, possess high stability and reproducibility, and should be easily fabricated in appropriate dimensions at a reasonable cost.

Electrochemical detection is very mass sensitive. Many analytes, including many endogenous neurotransmitters or neuroactive compounds, are natively electrochemically active, which allows them to be measured by electrochemical detection. Electrochemical detection scales very well with reduced sample volume, making it amenable to miniaturization. Progress at Eksigent Technologies toward the development of a microfabricated electrochemical detection system will be discussed.

LAB-ON-A-CHIP WITH INTEGRATED CARBON MICROELECTRODES

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In recent years, electrochemical (EC) detection has become a well-established methodology for use in microfabricated lab-on-a-chip (LOC) analysis systems. The focus of our group has been the use of conventional microfabrication techniques to create fully integrated LOC-EC devices in which all electrodes are incorporated directly onto the chip during the fabrication process. To date, such on-chip LOC-EC devices have usually employed platinum EC electrodes because this material is well suited to these microfabrication procedures [1]. However, to facilitate more diverse applications, it would be useful to have access to a wider variety of electrode materials, in particular, carbon.

This presentation will address our effort to make a LOC device made completely of carbon electrodes. In order to reach this goal, three approaches have been studied. Initially, carbon films were deposited by means of both e-beam (EB) evaporation [2] and direct current (DC) sputtering techniques [3]. The properties of the resulting electrodes, in terms of thickness, adhesion, and conductivity, were characterized along with their voltammetry with several model redox systems. Although the carbon films resulting from the sputtering technique did not prove to be suitable electrodes, carbon films obtained by the EB evaporation presented reasonable electrochemical properties. Finally, carbon electrodes were patterned onto fused quartz substrates by means of pyrolysis of photoresist [4, 5]. By choosing the appropriate type of photoresist and adjusting the parameters of application of photoresist, as well as pyrolysis conditions (time and temperature), carbon films with thickness varying from 0.5 to 1 micron were obtained. These carbon electrodes presented electrochemical properties analogous to glassy carbon electrodes. The top of chip and the channels, were patterned onto soda lime glass.

A low temperature, stamp-and-stick bonding technique was developed for assembly of the final LOC device. Several applications of the resulting chips will be demonstrated.

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MONITORING TRACE LEVELS OF HEAVY METAL IONS IN BIOLOGICAL FLUIDS USING ANODIC STRIPPING VOLTAMMETRY AND A BORON-DOPED DIAMOND ELECTRODE

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High concentrations of heavy metal ions (e.g., Cd^{2+} , Pb^{2+}) in the human body can lead to numerous health problems. Measurement of these toxins in biological fluids (e.g., blood and urine) can be used as an indicator of chronic exposure or a single incidence of a high level exposure. Therefore, it is important to have available analytical methods that can sensitively, reproducibly, and stably measure these contaminants in biological fluids.

Anodic stripping voltammetry is a useful method for monitoring heavy metal ions as the method generally provides a wide linear dynamic range, low limit of detection, and multimetal analysis capability. We have demonstrated that boron-doped diamond is a suitable electrode for metal ion analysis in water, soil, and sludge samples. Herein, we report on the use of this electrode and method to analyze metal ions in biological fluids. Direct analysis in urine simulant is possible; however, blood contains many biomolecules (e.g., proteins and peptides) that can adsorb on the electrode surface and inhibit the response. We report on the detection figures of merit for analysis of Cd^{2+} urine simulant and preliminary approaches to prevent electrode fouling in analyses of Pb^{2+} in blood samples.

OPTIMIZING MULTILINE ANALYSIS IN ICP-AES BY USING DEDICATED INTERACTIVE ASSISTANCE TOOLS INCLUDING SPECTRUM FILTERING AND DISPLAY, AND OUTLIER REJECTION

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The aim of any analytical system is to provide highly accurate results in quantitative analysis. In ICP-AES, use of multiline analysis is an efficient way to enhance reliability of the concentration results. Besides an efficient use of the information emitted by the ICP, that is, 165–800 nm spectra, the use of several lines per element allows the analyst to verify possible outliers due to unexpected interferences, and to increase

the concentration range. Although any system equipped with a solid-state multichannel detector can perform multiline analysis, this is not sufficient to obtain reliable element concentrations. Dedicated tools are necessary to take full benefit of the multiline analysis potential and to facilitate the task of the analyst. HORIBA Jobin Yvon has, therefore, developed several interactive assistance tools to be used with a CCD-based ICP system. Multiline selection is then facilitated by using a proprietary ICP-based spectra data base containing not only wavelengths, but also sensitivity and line width. The base is used through a filtering procedure to select lines with appropriate sensitivity and free from spectral interferences. An interactive display tool combines single-element spectra as a function of the expected composition of the sample. It is, then, possible to visualize the selected analyte lines and their vicinity within the matrix environment for a final validation. Moreover, because the display tool can include blank spectra, it can also be used to select background corrections. Once concentration measurements for each line have been obtained, an ANOVA-based statistical data processing tool is used to verify possible outliers. At the end, a reliable, single concentration per element is given.

RAPID AUTOMATED OPTIMIZATION OF A EUROPEAN PHARMACOPOEIA MONOGRAPH METHOD FOR ANALYSIS OF OLSALAZINE SODIUM BY HPLC

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Olsalazine sodium is used as one of the raw materials for a commercial product of a major international pharmaceutical company. The European Pharmacopoeia provides a monograph method for the quantitation of olsalazine sodium and known impurities by reversed phase HPLC. However, the early generation silica column specified in the method is prone to stationary phase degradation at the prescribed pH. Additionally, this method has an excessive total assay time of 55 minutes. The company's analytical R&D department was tasked with developing an improved HPLC method that would (i) be able to resolve and quantitate olsalazine sodium and all nine known impurities, (ii) have an acceptable total assay time, and (iii) perform with the robustness required for normal use as a quality-control method. The work presented describes how these goals were successfully accomplished using a fully automated HPLC method development experimentation software platform that had integrated multiple column and solvent switching and DOE-based experimental design, creation, and implementation capabilities. This software also enabled the determination of overall method robustness using a built-in robustness calculation algorithm. Graphical response surface graphs indicated significant interactions between the method study parameters, and the resultant method, generated by the automated optimizer, indicated that complete resolution of the olsalazine and all nine impurity peaks could be achieved within 34 minutes at a flow

rate of 0.5 mL/min and a final organic solvent concentration of 40%.

THE IMPLEMENTATION AND INTEGRATION OF DATABASING FOR INCREASED EFFICIENCY OF CHIRAL CHROMATOGRAPHIC METHOD SCREENING AND DEVELOPMENT

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Chiral chromatographic method screening proves to be an effective tool for rapidly developing chiral separation methods. We have established in our laboratory SFC, normal phase HPLC, and reversed-phase HPLC chiral screening tools, which provide >90% screening success rate and generate >500 chiral separation methods per year for chiral analysis and preparative chiral purifications in support of drug discovery and pharmaceutical process research.

To explore the opportunities to further increase the efficiency of chiral chromatographic method screening and development, an in-house chiral applications database was developed, implemented, and fully integrated with the screening workflows. The database software allowed us to streamline several tasks, including archiving sample and structure information, importing chromatographic data from various chromatography platforms, inputting screening results, and generating screening reports. Practical implementation of the databases to assist chiral method development is also being evaluated, with particular emphasis on searching strategies. The in-house chiral applications database and a commercially available database of chiral methods are searched simultaneously for prediction of chromatographic separation conditions with new pharmaceutical compounds. Searches were based on chemical structure similarities, derived from purely structural considerations as well as predicted physicochemical parameters. This approach on the creation and effective mining of previously collected data in order to more accurately target screening experiments will potentially reduce the experimental space examined to areas that have the highest probability of success.

ANALYTICAL CONTROL SYSTEM TECHNIQUES TO SUPPORT EVOLVING TEST EQUIPMENT MARKET

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Changes within the laboratory and test and measurement equipment industry are driving technical changes at the control system level. Changes such as manufacturer consolidation and the increased use of traditional test and measurement equipment within integrated manufacturing environments have created new control system issues. As a result, a

different control system approach is now required in order to effectively meet product needs.

In addition, the companies that evolve as a result of consolidation also need to increase the flexibility and reuse capacity of currently developed products. Increasingly, they must rely on product line architecture approaches that enhance the integration and affordability of new technology platforms.

This paper specifically illustrates how changes in both business and deployment environments impact the underlying technology of test equipment and challenge the production of next-generation products.

It also examines new engineering techniques, such as value driven analysis (VDA) and architecture tradeoffs analysis methodology (ATAM) and how they can be applied to develop new products. This paper will show how, using ATAM, it is possible to evaluate both existing and new control system architectures against new equipment drivers such as reliability, speed, flexibility, connectivity, and enhanceability. This paper uses case studies to demonstrate the use of variability and commonality constructs to effectively support a disparate product line with a single underlying technology platform.

THE USE OF BIOINFORMATICS TOOLS PRIOR TO MS CHARACTERIZATION OF NEUROPEPTIDES IN NEWLY SEQUENCED ORGANISMS

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Identification of neuropeptides and hormones in brain tissues using conventional proteomics approaches is challenging. Normally, one of the first steps is to homogenize the tissue, greatly diluting the peptides produced in specialized cells. In other cases, the presence of other peptides and proteins interferes with the identification of neuropeptides. The challenge becomes even more pronounced when identifying neuropeptides from species for which genomic information or annotation of neuropeptide genes is scarce. In this case, verification of neuropeptide by direct sequencing becomes necessary.

We have identified over 36 neuropeptide genes in the honey bee by the combined use of homology searches using BLAST, searching for pertinent gene features such as signal peptide and presence of basic amino acids, as well as codon scanning for repeating patterns. This combined approach enabled the discovery of neuropeptide genes not identified by gene finding programs such as GENSCAN and FGENEH, and reconciled differences in prediction of the same gene by two or more gene predictors. In order to aid such approaches, we have also developed a statistical algorithm (NeuroPred) that predicts cleavage sites in insects, mollusk, and mammals and reports expected molecular masses with or without putative post translational modifications.

We are testing these approaches using several newly sequenced genomes. For example, the *Tribolium castaneum*

(red flour beetle) genome sequencing project by BCM-HGSC has reached annotation stage, with the second assembly already completed. By applying gene finding strategies already implemented in the honey bee, we are identifying neuro-peptide genes from *Tribolium castaneum*. Moreover, using NeuroPred, we predict proteolytic products of the predicted genes, thereby producing a list of most likely neuro-peptides to be confirmed by mass spectrometry.

OPTIMIZING CHROMATOGRAPHY BY USING HIGHER COLUMN FLOWS, SPLIT INJECTIONS, AND SURGE PRESSURE SPLITLESS INJECTIONS IN GC/MS

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Conventional chromatography follows the theory of the Van Demeter curve where the best separation is achieved for helium at a linear velocity of 20 to 50 cm/sec and low column flow rates of 1 mL/min. Several experiments have been run to test the effect of higher carrier gas flows by GC/MS. These elevated flow rates of 3 mL/min require fast-scanning speeds of 5 scans/sec or 1 618 μ /sec. The actual capacity for the stationary phase can be increased with these higher flow rates, allowing for the use of thinner films. Run times are shortened and final oven temperatures can be lowered, reducing the run time and column bleed. Another interesting advantage was observed in minimizing irreversible absorption of active compounds such as pentachlorophenol and organophosphorus pesticides by shortening the time spent in the analytical column.

Another parameter studied was the flow of the carrier gas through the inlet during injection of the sample. Surge pressure splitless injections force more of the sample into the column and minimize breakdown by reducing the stress of a flash vaporization in a constant temperature injection. A split injection does the same thing, although a more sensitive mass spectrometer is required.

No adverse effects were noted in precision, sensitivity, or separation at elevated column flow rates and surge pressure splitless injections. The split injection actually enhanced the separation of more volatile compounds like N-nitrosodimethylamine (NDMA) and pyridine. The target lists studied in this project were those in EPA methods 8270 and 525.

AUTOMATED SPECTRAL SEARCHING TO PROVIDE SPECIFIC FUNCTIONAL GROUP DETECTION CAPABILITY TO GC-TOFMS

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The use of automated searching of mass spectra for specific patterns allows the GC-TOFMS system to be used as a selective detection system for numerous types of com-

pounds. When applied to the full chromatogram, this automatic search capability may be used to identify chlorinated or brominated contaminants such as solvents or pesticides in foods or environmental samples. With the use of GCxGC-TOFMS, trace components with specific functionality can be identified. Sulfur-containing compounds such as pesticides or minor flavor ingredients can be identified in complex mixtures, such as flavoring mixtures. This work demonstrates the use of the GC-TOFMS system as a specific detector with ability to provide mass spectral identification of compounds containing specific groups.

PYROLYSIS GC/MS USED TO PROFILE CRACKING PRODUCTS OF BIO OILS

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As fossil fuel has become more difficult to obtain, there has been renewed interest in developing fuel from alternate sources. One alternative fuel source being investigated and used in parts of the world is oil from agricultural products such as corn, rapeseed, or soy. Unmodified, or straight vegetable oil can be used as fuel in adapted diesel engines. Unfortunately, because vegetable oil is more viscous than diesel fuel, as much as 10–20 times, it can cause problems in such engines over time. To create biodiesel, a fuel with a viscosity closer to diesel, oil can be modified. Thermally cracking, or controlled pyrolysis, of bio-oil decreases its molecular weight and is thereby effective in converting oil to a more useable fuel, like biodiesel. However, because of complicated pyrolysis mechanisms, different pyrolysis conditions yield different products. This poster describes the use of pyrolysis GC/MS by chemists to study various pyrolysis conditions and their effects on different vegetable oils. Chromatograms of pyrolyzed vegetable oils are compared to chromatograms of diesel fuel to determine optimal pyrolysis conditions.

USE OF GC/MS FOR SCREENING OF PESTICIDE RESIDUES IN FOOD

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Retention times and response levels for GC/MS analysis can vary significantly between instruments, or for an individual instrument depending on its condition. Quantitative analysis normally requires, as a first step, the analysis of analytical standards to verify chromatographic retention times and establish calibration curves.

With the introduction of the “positive list” system in Japan and the corresponding increase in the number of target pesticides to nearly 700, not only the preparation of analytical standards is expensive and time-consuming, but also availability of pesticide standards is frequently a problem.

In this study, the necessary parameters for quantitative analysis (retention times, response factors, and mass spectra) were standardized. Reliable semiquantitative analysis was demonstrated without the use of calibration standards. A database and supporting software were created for analysis of pesticide residues in food.

MATCHING SOFTWARE TOOLS TO CHALLENGING FORENSIC GC/MS ANALYSES FOR IMPROVED LABORATORY PRODUCTIVITY

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Use of gas chromatography and mass spectrometry (GC/MS) as a tool for forensic chemistry analyses can result in considerable volumes of data to review and evaluate (or interpret). With pressures to reduce evidence backlogs and meet deadlines, data review can create a bottleneck. Software tools developed specifically for forensic applications can streamline data review and reporting, and thus reduce or remove the data review bottleneck. Software that addresses specific tasks or sample types ensures that results and reports provide the best fit between the data and the reporting needs. The use of different software tools was evaluated using GC/MS for the following disciplines: forensic toxicology, fire/arson, and controlled substances. These software programs were compared against standard data processing software to ensure that the best fit for each section of forensic chemistry was provided. Analytical validation of the software was performed, to evaluate reliability, applicability, accuracy of results, and subjective ease of use. Specific benefits that resulted from the use of each program were quantified in terms of time saved by the analyst for reviewing and reporting data. The results include a matrix for effectively matching software applications to analytical challenges in order to improve the workflow within the forensic lab while still maintaining integrity and validity of results. Overall, it was determined that matching software programs to specific analytical tasks can reduce casework time and improve sample throughput.

DETERMINATION OF FATTY ACID METHYL ESTERS IN FOOD BY GAS CHROMATOGRAPHY CHEMICAL IONIZATION MASS SPECTROMETRY WITH AUTOMATIC DERIVATIZATION

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The analysis of fatty acid methyl esters (FAMES) characterizes the lipid fractions in food, making them one of the most important applications in food quality control. Although

most edible fats and oils contain primarily linear saturated fatty acids, branched fatty acids, mono-, di-, and higher unsaturated fatty acids can also occur. Additional information about lipid fraction can be obtained by determining the position of double bond(s) and geometric configuration cis/trans. Whereas trans isomers have adverse health effects (an increase risk of coronary heart disease), the cis isomers typically do not. In fact, the US Food and Drug Administration recently amended its regulations on nutritional labeling to include the amount of trans fatty acids in the nutrition facts panel of the food (US FDA 21 CFR Part 101, Section 101.62).

The most common analytical tool to measure fatty acids in food is GC analysis after methylation to improve their volatility. Two critical aspects of the global method need to be optimized: speed of analysis to maximize throughput and selectivity of GC method to ensure resolution and reliable quantification of cis/trans pairs.

In this work, a method for automatic methylation of fatty acids with KONIK ROBOKROM autosampler and analysis of the corresponding FAMES by the KONIK HRGC + MS Q12 system working in CI mode are presented.

Automatic derivatization parameters (reagent volumes, temperature, and time of derivatization, etc.) and GC-CI-MS parameters (temperature of the source and reactive gas type and flow) were optimized to obtain better selectivity in the quantification of cis/trans pairs. Quality parameters of FAMES analysis were established. Finally, GC-CI-MS was applied to trace level determination of FAMES in biological matrices (animal feed, dairy products, vegetable, oils and other processed foods) and results compared in terms of selectivity and sensitivity with the analysis by GC-EI-MS.

TRACE LEVEL HEADSPACE ANALYSIS USING VAPOR PHASE SWEEPING

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Static headspace systems offer a simple and robust method of introducing volatile organic compounds into a GC. However, one of the drawbacks of static headspace analysis is that the technique cannot provide the sensitivity necessary to detect compounds at trace levels since only a portion of the equilibrated headspace vapor can be injected. Trace detection analysis generally requires much larger volumes of headspace vapor and the ability to preconcentrate these compounds of interest prior to GC analysis.

This paper will present the analytical advantages of a static headspace system equipped with a dual sampling needle capable of dynamically sweeping the headspace and concentrating the compounds prior to injection. The sensitivity of this type of headspace sampling is now proportional to the total mass of the compounds present in the vapor phase rather than equilibrium concentration of the headspace.

The advantages of this sampling method will be demonstrated by presenting sub-ppb-level results in a variety of condensed matrices. The full evaporative technique will also be evaluated since the dynamic sweeping injection technique is capable of creating enough headspace volume to overcome sensitivity issues associated with very small sample sizes.

A NEW AUTOSAMPLING SYSTEM FOR THERMAL DESORPTION GC/MS ANALYSIS OF CHEMICAL WARFARE, ENVIRONMENTAL, AND FOOD VOLATILES

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Volatiles sampling using sorbent technology has permitted sensitive and automated analysis of compounds from a wide range of sample matrices. By collecting the organics from a large sample volume and then thermally desorbing them into the GC/MS, parts-per-billion and even parts-per-trillion level analyses are possible. Selection of sorbent type and tube size helps in the development of techniques specific for a particular type of compounds or sample matrix. Consequently, the same technology may be used for the extremely demanding analysis of chemical warfare volatiles in air, industrial hygiene/environmental contaminants such as TICS, TIMS, solvents and combustion products, and even aroma compounds from foods and packaging.

This paper presents data on a unique system, comprised of a thermal desorption autosampler, a volatiles concentrator, and a GC/MS, which may be equipped with a variety of sample tube sizes to optimize the process for different applications. The tubes may be filled with different sorbent materials, and the desorption times and temperatures are independently controlled for each tube.

Application of the system is shown for several sample types, including typical surrogates for chemical warfare agents, aromatic and chlorinated solvents, and aroma compounds collected from the headspace of foods.

DEVELOPMENT OF ONLINE ULTRA VIOLET RADIATION PYROLYSIS-GC/MS (UV/PY-GC/MS) SYSTEM USING MULTIFUNCTIONAL PYROLYZER

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The physical and chemical performances of most polymeric materials gradually degrade due to external effects such as heating, photoradiation oxidative atmosphere, and mechanical stress. During the degradation process, not only the decomposed compounds formed from the sample but also the structural alternation of the samples has been important targets to analyze. By getting this information, it would be pos-

sible to prepare the advanced materials by modifying their molecular structures and/or selecting appropriate additives. For these analyses, a new analytical method has to be developed.

In this work, a new analytical instrument using an on-line micro-ultraviolet (UV) radiator combined with the multifunctional microfurnace pyrolyzer (PY-2010iD, Frontier Lab) with capillary column GC/MS was developed. A UV beam was spotted on a small amount of polymer sample set in the pyrolyzer through a fiber cable. The evolved gas from the irradiated polymer sample was analyzed online by GC, and then the residual polymer sample was pyrolyzed in the pyrolyzer to give a specific pyrogram. Based on both pieces of information obtained, the deterioration mechanism of the polymeric material during irradiation and the effect of additives such as photostabilizer and UV-absorber can be evaluated using submilligram order of minute polymer sample with relatively short test period compared with that by conventional technique such as a weather meter. Here the basic performance of this system was examined using typical polymer materials such as polystyrene and polypropylene.

AUTOMATION AND OPTIMIZATION OF LIQUID-PHASE MICROEXTRACTION

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Many fully automated liquid-phase microextraction (LPME) techniques, including static headspace LPME (HS-LPME), exposed dynamic HS-LPME, unexposed dynamic HS-LPME, static direct-immersed LPME (DI-LPME), dynamic DI-LPME, and hollow fiber-protected LPME (HF-LPME), are described in this study. All steps of these LPME techniques, including the filling of the extraction solvent, sample transfer and agitation, withdrawing the solvent to a syringe, and introducing the extraction phase into the injector, were autoperformed with a commercial CTC CombiPal autosampler. Critical experimental factors, including temperature, choice of extraction solvent, solvent volume, plunger movement rate, and extraction time, were investigated. Among the three HS-LPME techniques that were evaluated, the exposed dynamic HS-LPME technique provided the best performance, compared to the unexposed dynamic HS-LPME and static HS-LPME approaches. For DI-LPME, the dynamic process can enhance the extraction efficiency and the achieved method precision is comparable with the static DI-LPME technique. The precision of the fully automated HF-LPME is quite acceptable (RSD values below 6.8%), and the sensitivity of this method is comparable with the DI-LPME approaches, although only part of the extraction phase is introduced for analysis. The fully automated LPME techniques are more accurate and more convenient, and the reproducibility achieved eliminates the need for an internal standard to improve the method precision.

GAS CHROMATOGRAPHY/TANDEM MASS SPECTROMETRY FOR THE SIMULTANEOUS DETERMINATION OF PERSISTENCE ORGANIC POLLUTANT IN KUWAITI WASTEWATER TREATMENT PLANTS

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A method was developed to determine trace concentrations of individual PAHs and OCPs in sewage sludge samples, which were taken from three different wastewater treatment plants (WWTPs) in the area of Kuwait. The new method was based on using GC-ion trap MS techniques for the simultaneous determination of OPCs and PAHs, and the sludge samples were extracted with accelerated solvent extraction (ASE) using 1:1(v/v) dichloromethane (DCM): n-hexane solvent mixture. The extracts were passed through a multilayer column containing silica/Al₂O₃, followed by gel permeation chromatography (GPC) column in order to eliminate the large interference caused by organic compounds and lipids. Sulfur was treated by a nondestructive method such as using copper bars which enhance the removal of sulfur.

GC-ion-MS-MS optimization of several variables such as CID, ESL, and waveform was done perfectly in order to obtain exact fragment ions for each analyte and enhance the sensitivity of interested analyte, which was in the range of ppb. However, the correlation (R²) of the calibration curves of the analyte was exceeding 0.999.

Average concentrations of 7.8 mg/kg for the sum of 16 PAHs, and 39.5 µg/kg of the sum of the 16 OCPs were found in the sewage sludge. Compared to a previous study performed in our laboratory using a different analytical method, PAHs are still present in similar concentrations, and there is no evidence of large deviation from the results obtained previously, except that the linearity of the calibration curve was narrowed using ion trap techniques.

OPTIMIZATION OF GRAZING ANGLE ATTENUATED TOTAL REFLECTION FOR THE ANALYSIS OF MONOMOLECULAR LAYERS ON SILICON

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Interest in the analysis of monomolecular layers on silicon has grown significantly with the development efforts for thin dielectrics in the semiconductor industry. The need now exists to probe layers tens of nanometers in thickness reliably. Traditional FT-IR sampling techniques such as transmission or ATR typically measure samples or coatings on samples where the thickness is 3 orders of magnitude greater. Grazing angle reflection techniques have been used successfully to measure monomolecular layers on reflective substrates. However, in the case where silicon is the substrate, resolv-

ing the distinctive features of the surface film becomes difficult. As early as 1966, Harrick saw the need for the analysis of thin films on another substrate by ATR and provided detailed theoretical description of the phenomenon. Harrick's publications further described equations for calculation of effective thickness of very thin films on a base layer measured at parallel and perpendicular polarization. Later, Olsen and Shimura proposed an ATR method for the analysis of thin layers on silicon at 60 degrees using a germanium (Ge) crystal, and a theoretical absorbance amplification of 3 orders of magnitude relative to traditional measurements. Recently, Milosevic et al. further described a method of grazing angle single reflection ATR analysis using 60- and 65-degree Ge crystals for organic monolayers on silicon. This publication described theoretical detail for the enhancement in sensitivity relative to traditional ATR sampling of bulk materials and provided examples of spectral data for the analysis of organic monolayers on silicon using this optical configuration.

ACKNOWLEDGMENT

Efforts by the authors to reproduce these measurements have been partially successful and this has led to the current effort to detail an optimization of the configuration of a grazing angle ATR accessory for the FT-IR analysis of monomolecular layers on silicon.

MONITORING MANUFACTURING PLANTS USING PORTABLE ANALYZERS WITH GPS AND WIRELESS DATA TRANSMISSION SYSTEMS

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A new 4-channel analyzer (photoionization detector (PID), and three other sensors), model 102+, was introduced by PID analyzers last year. This analyzer has the capability of logging 7 000 points for each channel. The data can be downloaded to a PC via the RS232 and the software that is provided.

With a geographic position sensor (GPS) incorporated into this analyzer, the user could easily generate a concentration profile of the contaminants in the work area, and the data would be invaluable. If, in addition, we interface a wireless mote to this analyzer, we have the ability to transmit the data directly to a PC for database archiving and real-time monitoring.

Radiofrequency (RF) wireless "motes" are low-power, inexpensive devices that support internal and external sensors, processing, and communications in a small package. The "field mote" receives data from the model 102+ then relays it back to an RF "base mote" connected to a server with an MySQL database. Multiple motes, each with a unique ID number, can be used in the system with one mote in each analyzer.

The user can select from more than nearly 40 different sensors and technologies such as PID, IR, TCD, CG,

capacitance, temperature, and electrochemistry. This technology can be used to develop concentration profiles before and after ventilation is added, monitor areas where chemicals are being loaded or unloaded, monitor hoods because of the compact size, perform continuous monitoring during process operations, and so forth. And with the on-board GPS, if the system is moved, the new location is automatically known. Additional applications and data will be discussed.

CALIBRATING MERCURY CEM INSTRUMENTATION

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The clean air rule [1] has provided the legislation for the regulation and control of mercury emissions from coal-fired utilities in North America. To ensure the integrity of the systems designed to measure the mercury levels emitted, it is essential to develop suitable calibration sources which are traceable to national standards, be it the National Institute of Standards and Technology or other national standards bodies.

Since the introduction of its first mercury continuous emissions monitor, P S Analytical has provided a calibration source, the CAVKIT, which allows a set concentration of mercury to be introduced at various stages in the sampling and measurement cycle. This has been successfully used to check calibration and to test the integrity of the full system. The system has been evaluated by NIST [2]. Further developments to provide a source of oxidized mercury and the experience of this in the field will be described.

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DETERMINATION OF HEAVY METALS IN PRODUCED WATER AND DRILLING DISCHARGES USING ATOMIC FLUORESCENCE SPECTROMETRY

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During the extraction of oil and gas, a large quantity of undesirable water and drilling waste is produced. The chemical composition of discharged wastes is complex and may vary at different stages of oil and gas production. Drilling mud formulations have varied greatly over the years and are also dependent on the rock formation to be drilled. Barite (BaSO_4)

is commonly used in water-based drilling mud to control and regulate the hydrostatic pressure in the well. This material contains high levels of heavy metals such as mercury, arsenic, *selenium*, lead, cadmium, zinc, copper, chromium, and others. The cuttings of the drilling process are typically discharged to the environment giving cause for concern about marine wildlife. The environmental effect of heavy metals from drilling discharges has created much debate. Barite and associated trace minerals are of a nonsoluble form and are therefore believed by many not to be biochemically available to marine organisms.

This paper will discuss the analytical methods that can be used for determinations of heavy metals from drilling mud, discharges, produced water, marine sediments, and water. The paper will focus on the use of vapor generation atomic fluorescence spectrometry (AFS) as a method for determining mercury, arsenic, *selenium*, and antimony. This approach has been coupled to HPLC separation techniques to provide speciation measurements. A fingerprinting method using the trace metals of barite will be presented that potentially can be used to monitor the fate and distribution of heavy metals from drilling discharges.

THE ANALYSIS OF MERCURY SORBENT TRAPS BY DIRECT COMBUSTION AND COLD VAPOR ATOMIC ADSORPTION ANALYSIS

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On March 15, 2005, the Environmental Protection Agency (EPA) issued the first-ever federal rule to permanently cap and reduce mercury emissions from coal-fired power plants. In order to comply with the EPA mercury emission standard, the power industry is required to determine the amount of mercury emitted from their power plants. For this purpose, one approach may utilize continuous emission monitors, which are designed for the continuous sampling of total mercury emissions in flue gas streams. However, this approach is presently unproven and costly. Another method, referred to as EPA part 75 appendix K, allows for sampling of the stack effluent and offline analysis of sorbent traps. Procedures proposed for the analysis of the sorbent traps include extraction methods, draft EPA method 324 and draft modified ASTM method 6414, a thermal desorption method with cold vapor atomic absorption (CVAAS), and thermal decomposition CVAAS.

The sorbent trap analysis work described here utilizes the direct combustion methodology based on ASTM method D 6722, total mercury in coal, and coal combustion residues by direct combustion analysis. The sample preparation and handling methodology were modified so that the sorbent traps can be reliably and routinely analyzed. All analyses were obtained using a LECO AMA254 mercury analyzer. The resulting methodology has been shown to provide excellent

recoveries, often approaching 100%, as well as unprecedented accuracy and very precise mercury results.

The proposed combustion methodology will be described in detail. This method will be compared and contrasted with alternative approaches. Round robin results will be presented which utilize this direct combustion method on a set of preloaded sorbent tubes. Analyte recoveries, accuracy, and precision obtained in this study will be provided and these results will be compared to those obtained by competing methods.

DETERMINATION OF MERCURY AND OTHER TRACE ENVIRONMENTAL ELEMENTS USING MORE SENSITIVE METHODS AND ATOMIC FLUORESCENCE SPECTROSCOPY

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Mercury has long been known to be a potent toxin but recently concern has increased significantly about chronic high-level exposure to Hg and other hazardous metals found in the environment leading to health safety concerns. Recognizing this hazardous mercury pollution, many environmental agencies have published advisories against this contamination and issued stricter methods of analysis for mercury determination.

Recently, a simple fluorescence technique of EPA method 245.7 (mercury in water by cold vapor atomic fluorescence spectrometry) was developed to meet the increasing need for accurate, low-level mercury and other trace environmental sensitive elements. The advantages of this technique include low noise, ultra lower detection limit (e.g., it can be as low as 0.001 ppb for mercury), wider working range (10^3), and lower interference.

Aurora Instruments Ltd. has introduced a new generation of atomic fluorescence spectroscopy; AI 3300 offers a unique vapor generation with ppt detection limits for Hg and other hydride forming elements. With dual channel simultaneous measurement, the sample can be digested in one batch and both elements (Hg plus one of the other hydride forming elements) can be measured simultaneously. Moreover, the AI 3300 can be combined with the XYZ autosampler to give a flexible, fully automated operating system, which significantly increases the productivity and reduces the labor cost.

BRINGING TOGETHER US EPA METHODS 1631, 245.1, 245.7, AND 7473 FOR THE DETERMINATION OF MERCURY IN SOILS AND LIQUIDS

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Mercury determinations are required in a variety of sample matrices and across a very wide range of concentrations. Sen-

sitive techniques, such as purge and trap cold vapor atomic fluorescence spectroscopy, are available that can achieve detection limits at sub part-per-trillion sublevels but which cannot handle higher concentrations without massive dilutions. On the other hand, there are less sensitive techniques, such as direct combustion mercury generation that can handle diverse matrices with little to no sample pretreatment.

Instrumentation designed for the differing techniques shares much in terms of their detection systems but little in terms of sample introduction. In all cases, free gaseous mercury is the species that is ultimately quantified. The processes to produce the mercury gas, however, diverge significantly. Methods 1631, 245.1 and 245.7 employ chemical reactions with acids, oxidants, and reductants. Method 7473 employs sample heating followed by vapor catalysis.

We will introduce a combustion-based instrument to determine mercury in matrices such as solids, tissues, coal, and soils without sample pretreatment that can be equipped with a high-sensitivity liquid introduction system.

ADVANCES IN THE DETERMINATION OF MERCURY BY THERMAL DECOMPOSITION, AMALGAMATION, AND COLD VAPOR ATOMIC ABSORPTION SPECTROSCOPY

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US EPA anticipates increased implementation of method 7473 for the determination of mercury in a variety of sample matrices. The method provides rapid analysis with little to no sample pretreatment. In most cases, results can be obtained in less than ten minutes. Moreover, available instrumentation for this technique is remarkably stable and immune from matrix interferences. As a result, a variety of samples can be analyzed for mercury from a single stored calibration curve.

In this presentation, we will investigate the impact of system characteristics such as moisture control, gas selection, and decomposition temperature on accuracy, sensitivity, and stability. Using aqueous calibration standards mercury concentrations will be reported for a variety of certified reference materials.

AT-LINE, ONLINE, AND INLINE SAMPLING FOR NEAR INFRARED AND MID INFRARED MEASUREMENTS

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In moving an analysis from the laboratory to the process line, the spectroscopy stays the same but just about everything else changes. In particular, the analyst is no longer in control; the requirements of the production process almost always take precedence.

We designate at-line as close to but not connected to a process, online as connected with a slip stream, and inline as an installation inserted directly. Except in the at-line case, sample modification is usually impractical; the sample must

be dealt with as it is and even then sample handling must usually be kept simple.

The process may impose sampling restrictions that make one or the other spectral region the obvious choice. In other cases, either spectroscopy might be acceptable. The choice then will depend on other aspects of the analysis situation such as the following.

- (i) Is this a standard analysis available as a complete solution?
- (ii) What is the operator's skill level?
- (iii) What mechanical packaging is required?
- (iv) Is data communication necessary?
- (v) Will there be adequate service support?
- (vi) Price.

In other cases, spectroscopic variables may dictate the choice of spectral region. Some are as follows:

- (1) The convenient or required pathlength is
 - (i) long: use nonfundamental (weak) absorptions,
 - (ii) short: use fundamental (strong) absorptions.
- (2) Concentration of the component to be measured is
 - (i) trace: use fundamentals,
 - (ii) major: use nonfundamentals or very short path (e.g., ATR).

The sample stream is chemically aggressive; optical materials that are suitable for use at longer wavelengths may be vulnerable to be attacked by aggressive chemicals. At wavelengths shorter than $5\ \mu\text{m}$ ($2000\ \text{cm}^{-1}$), quartz, sapphire, and cubic zirconia are almost universally applicable.

The sample stream temperature varies; combination bands that involve very low frequency absorptions may be very temperature dependent. ATR measurements may be temperature-dependent due to refractive index changes. The amount of conditioning or compensation required will depend on the strength of the temperature dependence.

The sample stream pressure varies; pressure broadening in gases can cause changes in apparent absorption that are different for different bands. Hydraulic hammers in liquid streams can cause deflection of optical components. Pressure conditioning requirements will vary on a case-by-case basis but may be less severe if the wavelength region is carefully chosen.

DATA HANDLING FOR NIR SPECTROSCOPY AND PAT

Howard Mark

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The FDA has defined a set of goals for the pharmaceutical industry, called the Process Analytical Technology initiative (PAT). This initiative is intended to address the increasing pressure it is being subjected to which from Congress and the public to reduce the costs of pharmaceutical preparations. Instrumental methods of analysis such as NIR play a key role in addressing the new paradigm the FDA is promoting. Making those analytical methods conform to the strict

requirements of a regulated environment, however, leads the spectroscopic community into new territory and ways of doing things.

The success of modern NIR spectroscopy has depended, from the beginning, on the confluence of three distinct concepts: the extremely good precision and stability of modern instrumentation, the use of diffuse reflectance measurements to collect the spectral data, and the application of sophisticated multivariate methods to extract the useful information from the data. The advent of the new PAT initiative from the FDA has increased the need for new and innovative approaches to extracting information and simultaneously imposed new requirements on them. In this talk, we will examine the historical bases for analysis of NIR data, a new paradigm that has appeared, and the expectations for advancing into the future.

APPLICATIONS OF IMAGE ANALYSIS IN FORENSIC SCIENCE

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Image analysis (IA) is used in forensic science for comparative analysis between a known sample (control) and an unknown sample. The FDA's Forensic Chemistry Center (FCC) uses IA in cases related to counterfeit pharmaceuticals and illicit drugs. The purpose of this presentation is to describe a computerized IA method for comparing alpha-numeric printing encountered in evidence related to cases of counterfeit pharmaceuticals and illicit drugs. Because of the adaptive features of the human eye, the manual, real-time image analysis method is less sensitive to variations of shades of color and detail. The computerized image analysis method described in this presentation does not suffer from this subjectivity and therefore is better at determining subtle differences between samples. To achieve consistent results, the lighting procedure, the image acquisition, and the image analysis method were optimized. To minimize variations due to lighting, a lighting diffuser was used. To evaluate the computerized IA method and to assess the details revealed in an image comparison, computerized analysis of images was compared with manual image analysis at two different magnifications. Using digital scanner camera, low-magnification images could be sampled at high resolution, thus making it possible to analyze larger printing areas. This presentation will describe the computerized IA method, compared to the manual method, and actual case studies using the computerized IA method.

ADVANTAGES AND SCOPE OF A MODERN AT-LINE ANALYSIS SYSTEM IN PROCESS MONITORING

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The monitoring of production processes uses physical parameters such as temperature, and pressure but chemical

parameters are also used for process control and optimization. To achieve constant product quality, analytical measurements are indispensable. Reliable monitoring requires content determination of components, auxiliary substances, or reaction products. At the same time, these determinations are used for process documentation and validation. The measuring systems applied in practice depend on the integration in the process and the position of the system; we find inline, online, at-line, or offline analysis systems, each of which has its advantages and justifications. Inline measurements are direct continuous measurements (e.g., pH value or conductivity), and online measurements are carried out in a bypass at high frequency, while offline measurements in the laboratory offer maximum flexibility. At-line systems are less well known although they offer numerous advantages. They are positioned directly at the production line and allow rapid and reproducible analyses of important process-related parameters. If the task consists in measuring different parameters in samples from different sampling sites, at-line measurements are more economical than online measurements and just as flexible and reproducible as laboratory offline analyses.

This presentation gives a detailed description and discussion of a commercially available at-line system. Thanks to its robust design, the system is suitable for the severe conditions prevailing in production plants and continuously supplies analytical results. External information can be fed into the system via the integrated I/O components and internally be used for parameter or measurement control. The integrated I/O components also serve to output analytical results and signals to the process control system and link the at-line system to the process environment. The control software includes exporting of the results to a LIMS or to external servers and offers additional functions such as remote control.

The wide scope of application of the described Process-Lab at-line system is discussed and practical applications from various industries are presented. The applications include etching baths from the steel industry, electroplating baths from the plating industry, and phosphatizing baths from the automotive industry plus examples from the food, paper, and pulp industries.

LUMINESCENT QUANTUM DOT FRET-BASED ENZYME ACTIVITY PROBES FOR SCREENING PROTEASE INHIBITORS

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The presentation describes the development and characterization of analytical properties of quantum dot-based probes for enzymatic activity and for screening enzyme inhibitors. The luminescent probes are based on fluorescence resonance energy transfer (FRET) between luminescent quantum dots that serve as donors and molecular acceptors that are immobilized to the surface of the quantum dots through peptide linkers. Peptide-coated CdSe/ZnS quantum dots were

prepared using a one-step ligand exchange process in which RGDC peptide molecules replace trioctylphosphine oxide (TOPO) molecules as the capping ligands of the quantum dots. The peptide-coated quantum dots were labeled with rhodamine to form the FRET probes. The emission quantum yield of the quantum dot FRET probes was fourfold lower than the emission quantum yield of TOPO-capped quantum dots. However, the quantum dot FRET probes were sufficiently bright to carry out quantitative enzymatic assays. The probes were used first to test the enzymatic activity of trypsin in solution based on signal changes of the quantum dot FRET probes in the presence of proteolytic enzymes. For example, exposure of the quantum dot FRET probes to 500 $\mu\text{g/mL}$ trypsin for 15 minutes resulted in 60% increase in the photoluminescence of quantum dots and a corresponding decrease in the emission of the rhodamine molecules when detached from the surface of the quantum dots due to enzymatic cleavage of the peptide molecules. The quantum dot FRET-based probes were used to monitor the enzymatic activity of trypsin and to screen trypsin inhibitors for their inhibition type and efficiency.

AUTOMATED METHODS FOR ONLINE MONITORING OF HALOACETIC ACID CONCENTRATIONS IN DRINKING WATER DISTRIBUTION SYSTEMS

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The haloacetic acids (HAAs) are a class of disinfection by-products (DBPs) formed during the chlorination of drinking water. Currently, the USEPA regulates five HAA species: monochloroacetic acid, dichloroacetic acid, trichloroacetic acid, monobromoacetic acid, and dibromoacetic acid. The five HAA species are called HAA5, and due to possible health concerns, the USEPA has set a maximum contaminant level for HAA5 at 0.060 mg/L. Additionally, there are four unregulated HAA species commonly present in drinking water: bromochloroacetic acid, bromodichloroacetic acid, dibromochloroacetic acid, and tribromoacetic acid. These four unregulated species and the HAA5 species are called HAA9.

There are currently two methods approved by the USEPA for the determination of HAA9 in drinking water. The most recent method is USEPA 552.3, which is an update to USEPA 552.2. USEPA 552.3 uses liquid-liquid microextraction, followed by derivatization of the HAAs to their corresponding methyl esters and analysis by GC-ECD. USEPA 552.3 works very well for compliance monitoring (sampling on a quarterly basis) with superb method detection limit, accuracy, and precision values; however, when adapted to online monitoring, it becomes cumbersome at hourly sampling rates.

In this research, two analyzers and methods were developed for the purpose of online monitoring of HAA9 concentrations in drinking water distribution systems. The first analyzer is capillary membrane sampling-flow injection analysis (CMS-FIA) using the fluorescent reaction of nicotinamide with the HAAs (NCA-FL), and the second is

postcolumn reaction-ion chromatography (PCR-IC) using NCA-FL. The CMS-FIA uses a silicone rubber membrane within a Tefzel shell to separate the HAAs from the trihalomethanes (THMs), followed by reaction with NCA in basic solution and fluorescence detection of the NCA-HAA products. The CMS-FIA is able to analyze for total HAAs and total THMs, but it is not able to speciate within each class. The PCR-IC analyzer is able to speciate the HAAs in time using IC followed by postcolumn reaction with NCA to form the fluorescent NCA-HAA products. Method detection limit, accuracy, and precision studies, as well as two side-by-side online monitoring studies, will be presented for both analyzers. The monitoring studies were carried out in Memphis, TN, and Houston, TX, which directly compared CMS-FIA and PCR-IC to EPA 552.3.

MEMBRANE-BASED ANALYZERS FOR AUTOMATED ONLINE MONITORING OF TRIHALOMETHANE CONCENTRATIONS IN DRINKING WATER DISTRIBUTION SYSTEMS

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In the United States, chlorination is the primary drinking water disinfection process and is quite successful at preventing waterborne disease; however, it leads to the formation of disinfection by-products (DBPs). Since DBPs like the trihalomethanes (THM4) have possible health issues, they are a major concern for all utilities. The THM4 include chloroform, bromodichloromethane, dibromochloromethane, and bromoform. Presently, the maximum contaminant level (MCL) set by the USEPA for the sum total of THM4 is 0.080 mg/L. To conduct compliance monitoring, the USEPA has approved four gas chromatography- (GC-) based methods: 502.2, 524.2, 551, and 551.1. Even though they are very reliable with excellent method detection limit (MDL), accuracy, and precision values, they are only intended for quarterly or yearly sampling rather than hourly online monitoring. The development of automated, inexpensive analyzers/methods that can perform online monitoring of THM4 has gained interest due to the possibility of new rules and regulations requiring lower MCLs or better treatment strategies.

Three methods were developed: capillary membrane sampling-gas chromatography (CMS-GC), capillary membrane sampling-flow injection analysis (CMS-FIA), and online purge and trap gas chromatography (OPTGC). CMS-GC and CMS-FIA both take advantage of the CMS device, which uses silicone membrane tubing inside Tefzel tubing for extraction of THM4. OPTGC uses a gas extraction cell (GEC) that also contains silicone membrane tubing inline with an absorbent trap to sample then concentrate THM4. CMS-FIA cannot speciate THM4 as with CMS-GC and OPTGC, but can provide results for both total THM4 and total haloacetic acids (HAAs). CMS-FIA uses a fluorescence detector to detect the products from the reaction between THM4/HAAs

and nicotinamide (NCA) reagents in basic solution. OPTGC uses a dry electrolytic conductivity detector (DELCD) and CMS-GC uses an electron capture detector (ECD) to detect THM4. It should be noted that none of the developed methods are designed to conduct compliance monitoring or meant to replace the USEPA methods.

An overview of experimental details for these online monitoring analyzers/methods as well as a comparison of the advantages and disadvantages of each will be presented. The results from method detection limit (MDL), accuracy, precision, and two online monitoring studies conducted in Memphis, TN, and Houston, TX, drinking water distribution systems will also be presented.

QUICK INHIBITION MONITORING IN WASTEWATER TREATMENT PLANT OPERATIONS WITH A BENCH RESPIROMETER

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To an aerobic biotreatment system, changes including composition and concentration in influent can cause serious plant upsets due to inhibition effects. Quick monitoring and prediction of the impact of such changes are needed. This is especially critical to industrial wastewater treatment plant (WWTP) operations where the influent varies constantly and the operation is near capacity. Since the respiration rate is a direct indicator of activated sludge activity, respirometry can be an effective tool to monitor the WWTP performance. Currently, both online and bench respirometers have been used by many wastewater treatment facilities. However, due to its complexity, turnaround time, and resource demands, respirometry is not as widely used as other analytical methods, for example, TOC and COD. In this paper, we describe the use of a bench respirometer for quickly monitoring the influent inhibition to a WWTP. In the test, respiration rates were measured at different concentrations of wastewater. By comparing rates with the respiration of a control sample of activated sludge, the inhibition or toxicity as EC50 can be easily determined. The test usually takes less than one hour. This method was applied to map the influent ratio for multiple waste streams entering the treatment plant. It was also extended to assist a quick assessment of the toxicity of a single organic compound and a potentially new waste stream influent to the WWTP.

ENVIRONMENTAL MONITORING OF HYDROTHERMAL VENTS

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Hydrothermal vents along oceanic ridges were not discovered until the late 1970s, so much of their chemistry has yet to be elucidated. These black smokers reach temperatures of

up to 400°C and release a plethora of chemicals into their surrounding environment, especially sulfides. Studying these vents is of consequence because several different organisms utilize chemosynthesis as opposed to photosynthesis as they thrive off of the sulfides released. Raman spectroscopy is ideal to monitor different ions because of its ability to detect many compounds simultaneously, as well as the weak signal from water. In the present study, black smoker vents on the Juan de Fuca ridge in the Pacific Northwest were investigated using Raman spectroscopy. Signal enhancement was achieved through the use of silver nanoparticles.

HIGH-THROUGHPUT CHIRAL COMPOUND ANALYSIS

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Chiral compounds are increasing in interest due to their binding specificity, enabling directed targeting for drug interaction. It is the challenge of the organic and analytical chemist to facilitate their synthesis, purification, analysis.

The pharmaceutical standards placed on drug candidates require that these chiral compounds be fully resolved for characterization and the successful purification of the enantiomers also requires a baseline resolution factor. Analysis of chiral compounds can be time-consuming based on their limited resolution, R_s factor. There are several chiral columns which are targeted to the specific sigma and pi interactions of a chiral compound; however many compounds share many features related to the separation characteristics of multiple columns. The problem is usually that only one of these features is key to the resolution of the enantiomers, so the compound is generally run on several closely related columns to achieve the desired resolution of these enantiomers.

An application to address this time-consuming factor has been accomplished with the use of a custom configured HPLC system. The system is loaded with the samples of interest and each sample is automatically injected into a single port with a quaternary injection system that fills four simultaneous injection loops analytically. The sample is analyzed using a custom gradient profile and the software automatically generates and/or emails each sample's analysis by the four columns. This procedure represents a fourfold increase in productivity and is expandable to eight simultaneous analyses.

TECHNOLOGIES TO ACCELERATE ANALYTICAL THROUGHPUT ON A GAS CHROMATOGRAPH

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Many analytical laboratories use GCs for routine applications involving large numbers of samples. In such instances, sample throughput becomes an important operational aspect.

In this paper, we investigate various technologies for reducing the overall analysis time by shortening the time used

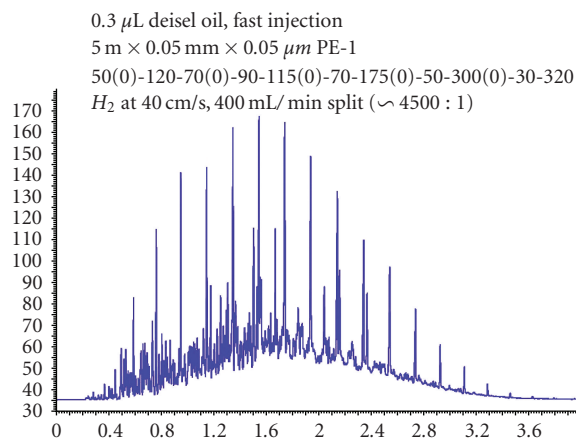


FIGURE 3

for the chromatography and reducing the “wasted” time in between chromatograms spent in cooling and equilibrating the oven and in preparing an autosampler to be ready for the next sample injection.

Examples are shown where the chromatography time is reduced by using high oven temperature programming rates in conjunction with short, narrow-bore, thin-film capillary columns using hydrogen as carrier gas. There are, however, tradeoffs to this approach and alternatives such as isothermal chromatography, column backflushing, and heart cutting are shown and discussed.

In many instances, however, the user will have an established method and will not wish to develop new conditions to accelerate the chromatography. In such instances, the time the system spends in coming ready for the next run becomes critical. A radical new GC air-bath oven has been developed to cool from 450°C to 50°C in less than 2 minutes. This is able to reduce the oven cooling time by several minutes over more conventionally designed ovens. This time reduction is further improved by the preemptive rinsing of the liquid autosampler syringe with the next sample prior to the GC becoming ready. In this way, significant time-savings are seen with no changes to existing analytical methods.

Figure 3 shows a chromatogram of diesel fuel run at fast temperature programmed rates with the fast cooling and the preemptive autosampler syringe rinse giving an overall cycle time of less than 7.5 minutes even though the column is being programmed from 50°C to 320°C, and prerun system checks such as automatic carrier gas leak testing and flame ionization detector flameout testing are applied.

SIMULTANEOUS DETECTION OF VOLATILE AND SEMIVOLATILE ORGANICS BY GC/MS

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Despite the advancements in available instrumentation, automated delivery equipment, and data analysis software, the

rate at which high-throughput laboratories can process samples is still limited by the complexity of the matrix. Fast gas chromatography (GC) with a variety of selective detectors has found wide application as a rapid screening technique for environmental samples, though most current applications illustrate the excellent separation efficiencies that can be achieved for the analysis of a small number of compounds. The analysis of complex mixtures has generally been avoided since the ability to provide positive target compound identification is compromised by coeluting target compounds and increased matrix background noise under fast acquisition conditions. In this study, fast temperature programming conditions and spectral deconvolution software were employed to develop a GC/MS method for the quantitative analysis of 105 widely different volatile and semivolatiles. The resulting 16-minute method was used to analyze fortified soil samples in the absence of a sample cleanup procedure, and the results were compared with those obtained by traditional Environmental Protection Agency (EPA) methods SW-846 8270 and 8260 analyses conducted by an independent contract laboratory. The results showed that while the standard EPA analyses failed to identify nine of the target compounds, the 16-minute GC/MS method with mass spectral deconvolution identified all 105 target, surrogate, and internal standard compounds with measurement accuracy well within the acceptable recovery ranges of the EPA method. It was therefore possible to increase the productivity of laboratories conducting environmental analyses by combining these two EPA methods into a single abbreviated GC/MS method using mass spectral deconvolution software. Productivity can be further increased as the use of spectral deconvolution also eliminates the need for time-consuming sample cleanup.

A HIGH-THROUGHPUT LC-MS/MS METHOD FOR THE DETERMINATION OF A-804693 (ABT-279) IN MOUSE PLASMA USING AN AUTOMATED 96-WELL PROTEIN PRECIPITATION

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This method utilized reversed-phase high-performance liquid chromatography (HPLC) with electrospray ionization tandem mass spectrometric (ESI-MS/MS) detection. Sample preparation was accomplished with an automated 96-well protein precipitation procedure. The HPLC separation was carried out on YMC HPLC column (ODS-AQ S-5, 120 Å, 2.0 × 150 mm) at a flow rate of 0.3 mL/min with a run time of approximately 5 minutes. ABT-279 was eluted using a mobile phase of 20 mM NH₄OAc in 20/80 (v/v) acetonitrile/water. A deuterated ABT-279, A-804693 D3, was used as an internal standard to account for the difference due to extraction recovery, ion suppression, matrix effects, and instrumental performance. Mass spectrometry detection was achieved using a PE Sciex API 3000 with triple quadrupole mass spectrometer equipped with a turbo ionspray as LC/MS interface.

ESI mass spectra were acquired in a positive ion mode with multiple reaction monitoring. Data were acquired and processed with analyst 1.3.2.

Since semiautomated sample preparation was employed with a short run time, 60 samples could be prepared in one 96-well plate and 120 samples could be analyzed per day per system. No interference was observed from matrix across the elution windows of 2.2 to 4.15 minutes, which indicates the specificity of the method. Acceptable assay precision (*D* 1.7% CV) and accuracy (bias between -8.4% and 2.1%) were obtained over a linear range of 21 to 21000 ng/mL. The mean correlation coefficient was 0.9980 ± 0.0003. The extraction recovery ranged from 46% to 52%. Since deuterated analog was used as an internal standard, no ion suppression was observed. The plasma samples went through 5 freeze/thaw cycles with no degradation.

This high-throughput method was robust, sensitive, specific, and accurate. This method has been successfully used to quantify ABT-279 in mouse plasma to support in vivo mouse micronucleus assays.

THROUGHPUT OPTIMIZATION OF A SCREENING SYSTEM BASED ON A CAPILLARY, SERIAL-LOADING PARALLEL MICROREACTOR

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Micro total analytical systems (μ TASs), mainly based on microreactor technology, have caught great attention in various analytical areas such as DNA analysis and drug discovery. Less commonly, μ TASs are used to explore properties of chemical reactions. The microreactor confers many advantages over conventional scale chemistry, including enhancement in heat and mass transfer, high surface-to-volume ratio, easy control of concentration gradients, and the possibility of eliminating unwanted side reactions. Other benefits also include safety and reduced exposure to toxic and hazardous materials. To date, the majority of microreactor systems are built up on a chip platform. These are effective for rapid reactions. Essentially, a single bolus of reactants is passed through the system and conversion to product is measured online. For slower reactions, this is impractical.

We have devised a parallel approach in which many boluses of reactants are injected serially into a capillary-based microreactor. The reactions, which may take hours, occur in parallel. Analysis is by online rapid GC or HPLC. The use of capillaries also eliminates the complicated fabrication process, reactors are easy to make, and temperature is easy to control.

Optimization of the reactors has been accomplished to yield the maximum number of reactions per unit time. The dimensions of the microreactor including internal diameter, length, and the residence time of solvent have been extensively studied. A mathematical model has been established based on Taylor dispersion theory. The maximum throughput of the reactor is in the range of 5- to 6-hour reactions

per hour. Our experimental system is the L-proline catalyzed aldol reaction of acetone and benzaldehyde.

REAL-TIME SCREENING OF APTAMER-PROTEIN INTERACTIONS BY PDMS MICROARRAY AND SURFACE PLASMON RESONANCE IMAGING

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Real-time screening of DNA aptamer-human Immunoglobulin E (IgE) interactions by surface plasmon resonance imaging (SPRi) is described. A multichannel PDMS flow cell was implemented, making it possible to perform affinity screening of biological interactions on one chip in a single run. Thiol-terminated aptamers were self-assembled in defined PDMS channels on a gold substrate. Cysteamine was codeposited with the thiol aptamers to maintain the aptamer binding efficiencies. Four aptamers with different nucleic acid sequences were tested for their interaction strength toward IgE and the result confirmed that aptamer-I (5'-SH-GGG GCA CGT TTA TCC GTC CCT CCT AGT GGC GTG CCC C-3') has the strongest binding affinity. Both positive and negative control experiments were conducted, showing a selective binding of the aptamer-I to the IgE molecules. A linear relationship between IgE and aptamer-I was obtained, and a 2 nM detection limit was achieved. Global fitting of the SPRi sensorgrams was successfully performed, and the dissociation constant of the aptamer I-IgE complex was determined to be 273.6 ± 0.8 nM, which agrees well with the reported values in the literature. The aptamer affinity screening by SPR imaging demonstrates significant advantages over competing methods because of the nonlabeling, real-time, and potentially high-throughput features. The capability of providing both qualitative and quantitative results on a multichannel chip shows that SPRi is a powerful tool for the study of biological interactions in a multiplexed format.

ELECTRONIC LABORATORY NOTEBOOK OR LIMS? HOW TO DECIDE?

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Laboratory Informatics has long promised to revolutionize the analytical laboratory, yet most laboratories today are characterized by isolated ships of automation captained by individual analysts navigating a sea of paper. Even the most automated techniques are reduced to printing paper reports for review and approval by analysts and supervision. The latest tools which are being touted to correct this problem and to achieve the paperless laboratory are electronic laboratory notebooks (ELNs).

This paper will address the role of electronic laboratory notebooks in the modern laboratory relative to established

informatics solutions like LIMS, as well as defining an architecture and decision tree for determining what solutions are best for their organization. Some of the factors to consider include

- (i) existing IT infrastructure;
- (ii) existing informatics solutions (LIMS, chromatography data systems, data repositories, document management solutions);
- (iii) type of laboratory (R&D, quality);
- (iv) end-user capabilities;
- (v) company and customer culture;
- (vi) regulatory constraints.

It is a mistake to assume that one size fits all or that a single informatics solution will meet all needs. Case studies will be discussed.

THE NEED FOR PROCESS SIMULATION IN LAB INFORMATICS INITIATIVES

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Mapping and analysis of workflows and definition of business requirements have long been recognized as absolute prerequisites for successful implementation of informatics systems in the labs. With the increased emphasis on the implementation and integration of new informatics tools like electronic lab notebooks (ELNs) and scientific data management systems (SDMSs) which are intimately intertwined with laboratory business processes, traditional process mapping approaches are no longer adequate.

There are now many options to consider and choices to be made on how to split up the scope of requirements between the new and entrenched systems. But which process is easier and more efficient for the lab analysts? Which is more secure? Which one is more cost effective? Add to this the fact that most organizations are now requiring hard process improvement metrics before approving budgets for these new informatics programs and it becomes obvious that the traditional approaches to static process mapping are inadequate in supporting these decisions. Instead, a tool like dynamic process modeling and simulation is required to model the complex interactions between process and new technology options and to provide budget approvers of the hard metrics about which they need to make funding decisions.

In this presentation, we will provide some background on what is involved in using a tool like process simulation for labs, some of its distinct advantages over conventional static modeling approaches, and we will present a lab informatics case study to show how modeling and simulation can provide meaningful benefits to lab informatics programs.

IMPLEMENTATION OF SAP IN THE QA/QC ENVIRONMENT: ONE ROLLOUT EXPERIENCE

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A rapidly growing number of corporations are moving their information structure toward a more centralized enterprise-wide model where all the key information about their business can be readily accessed with a few key strokes from anywhere in the world. From the purchase of raw materials through the delivery of the finished product, the availability of the data, facts, and figures is key to being able to compete in the fast-paced marketplace of today's business environment. The chemical industry is no exception.

Many of today's most competitive businesses have incorporated this type of centralized, enterprise-wide, multi-faceted information systems to handle all aspects of their business. One such system is SAP.

While the implementation of SAP can encompass all aspects of a business, the topic discussed here will be the implementation of the quality module of SAP into a quality assurance/control laboratory environment which already had 20 years of LIMS use and experience.

SAP and other enterprise-wide software systems, by their very nature, require a highly structured series of data entry steps in a rigid workflow in order to perform smoothly. Adapting such a rigid workflow was difficult in an existing, mature chemical business which (1) makes bulk chemicals for inventory (no customer identified initially) or (2) responds to customer requests which at times only provide 48-hour lead time. The case study presented here will describe one lab solution to these manufacturing scenarios incorporating the use of SAP for finished product data storage and reporting as a part of enterprise-wide strategy, and the continuing use of an existing LIMS for "in-process" testing.

MASS SPECTROMETRIC PROFILING OF NEUROPEPTIDE EXPRESSION AND SECRETION IN DECAPOD CRUSTACEANS

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Neuropeptides are a class of complex peptide hormones that are known to influence many essential physiological processes. As an ongoing effort to characterize these signaling molecules, we identify the neuropeptide complements of brain, thoracic ganglion, the sinus gland, and the pericardial organ from the lobster *Homarus americanus*. Several MS-based analytical methods have been employed, with im-

provement on small-scale sample preparation, such as direct tissue analysis and on-target digestion. To improve detection of higher mass peptides, dilute DHB was examined as matrix and a low amplitude SORI-CID burst was used to clean up matrix adduct. These improvements, coupled with the high mass resolution and accuracy of FTMS, enabled a complete profiling of a wide mass range of neuropeptides covering m/z 500–9000 in the tissue samples of the lobster *H. americanus*. Such peptidomic analysis was further enhanced by the de novo sequencing capability offered by LC-ESI-QTOF.

To study neuropeptides' functions, we also examine peptide hormones in circulating hemolymph, which is quite challenging due to low peptide concentration, interference from high salt concentrations, and abundant large proteins. To prepare the hemolymph sample, acidified methanol and ammonium citrate buffer was added to extract peptides and precipitate proteins followed by the use of a 10 K MWCO filtering membrane to remove big proteins. As a result, we identified several peptide hormones in the hemolymph samples. Furthermore, we investigate the effects of temperature acclimation on the hemolymph peptide profile change. Using green shore crab *Carcinus maenas* and lobster *H. americanus* as model systems, we explore the neuropeptidome changes in response to environmental stress such as salinity change and temperature variation. Both direct tissue samples and circulating fluids are evaluated and compared to identify potential neuropeptide players involved in regulating these physiological processes.

USING MASS SPECTROMETRY TO IDENTIFY PEPTIDES IN GLIA

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In the brain, glia are present at higher numbers than neurons, and yet have been much less studied. In the past decade, several studies have examined both the structure and function of astrocytes, a subtype of glia. Astrocytes express membrane ionic channels, transmitters, and receptors related to important signaling pathways in the brain. These discoveries have attracted scientific interest in the important role of astrocytes in cell-to-cell communication. The identification of neuropeptide-like molecules in astrocytes provides an additional means for such a communication between astrocytes and neurons. In this work, capillary liquid chromatography coupled offline with matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (CapLC-MALDI-TOF-MS) and TOF/TOF-MS have been used to identify peptides in astrocyte type-I cell lines from mouse cerebellum. These samples were extracted, and the extract was separated on a 3 μm diameter reversed-phase column at 2.5 $\mu\text{L}/\text{min}$. The separated fractions were deposited onto each sample spot on the MALDI target for mass spectrometric analysis. CapLC-MALDI-TOF-MS analysis detected multiple peptides, and the subsequent TOF/TOF-MS and

peptide sequence analysis allow their identification. Currently, additional peptides are being characterized. Next, putative functions will be evaluated based on similarities to known cell-to-cell signaling molecules. The preliminary results are consistent with the involvement of peptides from glia in cell-cell communication in the brain.

ACKNOWLEDGMENTS

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USING MICRODIALYSIS COUPLED ONLINE TO CAPILLARY ELECTROPHORESIS TO STUDY THE RAPID EFFECTS OF ESTRADIOL: A MECHANISTIC AND BEHAVIORAL APPROACH

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One way to study brain function is to correlate changes in neurotransmitter levels with behavior and pharmacological manipulation. In such measurements, temporal resolution is important in achieving good correlations because neurochemical concentrations fluctuate rapidly. In previous work, we have coupled microdialysis online to capillary electrophoresis and demonstrated that this method can measure changes in several amino acid neurotransmitters every 13 seconds, realizing a 60-fold improvement in temporal resolution over typical HPLC measurements of dialysate. As previously demonstrated, this improvement is made possible by the high mass sensitivity and automation of CE. In this work, we use this novel instrument to study the rapid effects of estradiol on neurotransmitter release. In the first experiment, we measure the effect of estradiol on gamma-aminobutyric acid (GABA) release. We have found that estradiol decreases GABA release and this release is further reduced through overexpression of the alpha estrogen receptor. This result supports the hypothesis that estrogen enhances dopamine function through the alpha estrogen receptor, therefore reinforcing addictive substances such as cocaine by inhibiting GABA release, which in turn disinhibits dopamine release. We are presently evaluating whether estradiol exerts this effect through a mitogen-activated protein kinase (MAPK) pathway. We are currently applying this mechanistic data to a behavioral model. In a second experiment, we are investigating whether a gender difference exists in glutamate and GABA releases after cocaine sensitization. A greater behavioral response is observed in sensitized females compared to males upon cocaine challenge. We are investigating whether an enhancement in GABA and/or glutamate is correlated to this enhancement in behavior and how hormones affect this release. These experiments will help elucidate sex differences in brain function in pathways important in addiction.

ONLINE, IN VIVO MICRODIALYSIS MONITORING OF EXTRACELLULAR LACTATE LEVELS DURING LONG-TERM ACTIVITY IN CEREBELLAR CORTEX

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Recent research on brain metabolism has taken a serious interest in lactate as a substrate for energy metabolism [1]. We have previously established an experimental model in rat cerebellum that has allowed us to examine interplay between neuronal spiking activity, synaptic activity, cerebral blood flow [2]. In order to extend this model to include online neurochemical monitoring we have developed a high-sensitivity version of our rapid sampling assay for online microdialysis [3]. This bioelectrochemical assay can now detect 2 micromolar concentration changes at 10-second sampling intervals. With this assay, we now monitor glucose, lactate changes in response to electrical stimulation in cerebellar region.

Short periods of electrical stimulation (2, 5, and 10 Hz for 30 seconds) were found to produce no discernable changes in glucose or lactate levels (despite increases in blood flow). Prolonged stimulation (5 Hz for 20 minutes) however yielded transient increases in lactate levels of 20–50 μ M. On cessation of the stimulus, lactate levels recovered transiently. Glucose levels remained unaffected throughout. With topical application of CNQX (ampa receptor blocker), the lactate responses to electrical stimulation disappeared and were shown to reappear with subsequent washout of the drug. With topical application of DAB (glycogen breakdown inhibitor), the lactate responses remained unchanged; however, blood glucose levels were shown to fall in response to the stimulations. These results will be discussed in terms of the role lactate plays as a metabolic neuronal substrate.

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IDENTIFICATION OF *TETRAHYMENA THERMOPHILA* PROTEINS VIA LC-MS AND LC-MS/MS

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Tetrahymena thermophila is a ciliated, unicellular protist that is often used as a model organism in biology labs due to its

ability to reproduce quickly and its nontoxic and robust nature. Interestingly, it has the ability to undergo asexual and sexual reproduction via a conjugation junction. The goal of this project was to identify as many proteins within the conjugation junction as possible. Using various isolation techniques, proteins were extracted from the conjugation junction of *T. thermophila* and then underwent SDS-PAGE followed by enzymatic digestion using trypsin. The tryptic peptides were injected into an HPLC equipped with a microbore C18 column, and then analyzed using an electrospray ionization (ESI) ion-trap mass spectrometer. ESI generated an assortment of multiply charged ions. Analysis of the spectra allowed for the identification of potential tryptic peptides, which were isolated and fragmented via tandem mass spectrometry. The resulting fragment ions were then screened against a *T. thermophila* database containing theoretical proteins based on its genome. Using the database, the identities and probable functions of the proteins could be determined. Proteins identified by the database were individually compared to the empirical data to validate that the results matched. So far, this project has led to the identification of 31 distinct proteins isolated from the *T. thermophila* conjugation junction.

ACKNOWLEDGMENTS

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DETERMINING TOTAL ANTIOXIDANT CAPACITY USING INLINE CAPILLARY ELECTROPHORESIS ASSAYS

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This work focuses on developing a rapid and reliable method to test for antioxidants using capillary electrophoresis. Antioxidants are important in preventing free radical damage to cellular components including DNA. Common antioxidants include ascorbic acid, catechins, and phenolic acids which are found in a variety of food samples and dietary supplements. Due to the difficulty in isolating and quantifying all the individual antioxidant components of a sample, total antioxidant capacity (TAC) has become an important measurement. TAC takes into account all the antioxidants in a sample as well as their relative oxidation capability. Current methods to measure TAC, including FRAP and ABTS, are both lengthy and labor intensive. Capillary electrophoresis provides automation, fast analysis, as well as minimal sample and waste volumes. This method relies on two sequential redox reactions occurring within the confines of the CE capillary. The first reaction involves the complete oxidation of the antioxidant(s) in the sample with an excess of N-bromosuccinimide. The remaining N-bromosuccinimide then reacts with a plug of excess ascorbic acid. The ascorbic acid that remains is detected using absorbance at 265 nm. The characterization and validation of this method, as well as its application to measure the TAC of a real sample, will be presented.

HIGH-THROUGHPUT LOW INTERFERENCE NUTRIENT ANALYSIS FOR SOIL/PLANT SAMPLES USING ADVANCED AUTOMATED DISCRETE ANALYZER AND CONTINUOUS FLOW ANALYZER

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Increased demand for soil testing and plant analysis is driven by nutrient management regulation and associated requirements for environmental monitoring and farmer awareness of benefits from regular use of soil/plant analysis. Regular soil testing and plant analysis eliminate expenditures on unnecessary fertilizers and prevent excessive rates and losses of required plant nutrients to surface and groundwater. Concentrations of nutrients, such as total N and NO₃-N, and total P and extractable P, and cations such as aluminum, magnesium, manganese, and potassium are used to evaluate plant health, soil and water quality, and management of cropping systems. Manual colorimetric methods are used for analysis of nutrient concentrations in plant, soil, and water samples. Time requirements and poor reproducibility of manual methods make it difficult to achieve goals for high precision and high throughput of samples during plant, soil, and water analysis. Interference of extracted sample matrix makes the analyses harder to get high-accuracy and low-interference data.

This presentation will demonstrate high-accuracy data by elimination of matrix interference using advanced diode array spectrophotometer in a discrete analyzer (model DA3500) and compare high-precision results with data generated with continuous flow analyzer (Model FS3100) available from OI Analytical, College Station. Analyses of nutrients, such as N, P, and cations in soil/plant sample, will be reported and evaluated from interference studies. The specific enhanced data quality and productivity benefits to soil/plant analysis will be presented. Advantages of new discrete technology and continuous flow technologies will be highlighted.

TRACE-LEVEL DETERMINATION OF CATIONS IN THE SECONDARY CIRCUIT OF A PWR-TYPE NUCLEAR POWER PLANT USING ION CHROMATOGRAPHY AFTER INLINE SAMPLE PREPARATION

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Thermal power plants consume very large amounts of water. Water works as the heat transfer medium and its expanding vapor drives the turbines to produce electricity. In order to prevent corrosion, the water has to be maintained at a pH above 7. In general, this is achieved by the addition of Lewis bases such as ammonia and ethanolamine. Besides the added chemicals, also corrosive compounds such as sodium, sulfate, and chloride can enter the water system of power plants. The

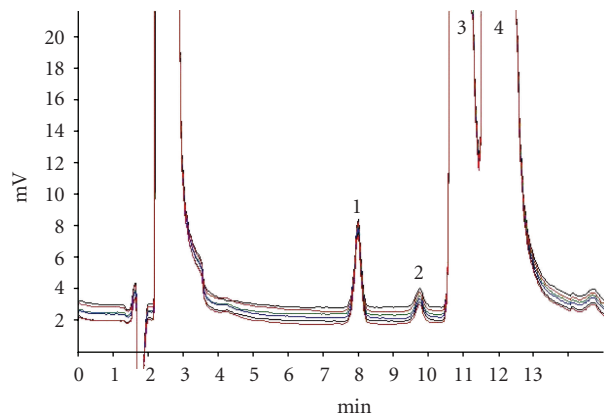


FIGURE 4: Ion chromatogram of a sample from the secondary circuit of a nuclear power plant. Peaks: (1) lithium, (2) sodium, (3) ammonium, and (4) ethanolamine (column: Metrosep C2 – 250; eluent: tartaric acid-dipicolinic acid eluent).

presence of these ions indicates intrusions of external cooling water or contamination problems due to condenser tube leaks.

An efficient water chemistry monitoring program controls both the added and the detrimental ions, thus preventing failures and extending the operating lifetimes of components in contact with water. Ion chromatography is the method of choice to meet the analytical challenge. However, the large concentration differences between the added bases and the alkali metal ions often complicate quantification and require time-consuming sample preparation steps. Whereas sample preparation steps used to be carried out before the sample entered the IC system, these steps have now been automated and included between the sample changer and the IC. A preconcentration column acts as the interface between the IC system and inline sample preparation. All sample preparation is automated and controlled by the instrument software. The cations to be analyzed enter the IC system via the preconcentration column. This arrangement is very robust, easy to configure, and highly suitable for accurately determining trace levels (ppt) of lithium and sodium in the presence of ppm quantities of ethanolamine (Figure 4). The relative standard deviations are better than 1.5%. Carryover for metal cations is below 0.3%; recovery rates are better than 98.5%.

HPLC METHOD FOR SIMULTANEOUS CELLULAR REDOX AND ENERGY STATE DETERMINATION OF PLANT SAMPLES

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An adaptation of an HPLC-based method developed by Lazzarino et al. (Analytical Biochemistry, 322 (2003) 51–59) was used for the determination of reduced and oxi-

dized ascorbic acid, reduced and oxidized glutathione, oxidized nicotinamide adenine dinucleotide (NAD), reduced NADH, oxidized nicotinamide adenine dinucleotide phosphate (NADP), and reduced NADPH. Sample preparation involved a series of three extractions, using acetonitrile, a 10 mM solution of potassium phosphate (pH 7.4), and chloroform. Samples were ground with glass beads prior to extraction. All samples were filtered before they are analyzed by HPLC. HPLC analyses were carried out using a Dionex Summit HPLC system (Dionex Corporation, Sunnyvale, Calif, USA) with a photodiode array detector (Dionex PDA 100). A Dionex C18 column (4.6 mm × 150 mm; 5 μm) and guard column were used. Column temperature was maintained at 18°C with a TCC-100 column compartment. Mobile phase A is an aqueous solution consisting of 10 mM tetrabutylammonium hydroxide (TBAOH), 10 mM potassium phosphate, and 0.125% methanol at a pH of 7.00. Mobile phase B is an aqueous solution of 2.8 mM TBAOH, 100 mM potassium phosphate, and 30% methanol at a pH of 5.50. A linear gradient from 100% phase A to 0% phase A and then back to 100% phase A was used over a 100-minute total run time. We are applying this methodology to better understand the effect of overexpression of genes involved in the myo-inositol pathway to ascorbic acid in the metabolism of antioxidants and energy-related molecules in *Arabidopsis* plant samples.

CHALLENGES AND CONSIDERATIONS FOR BUILDING AN AUTOMATED METHOD DEVELOPMENT SYSTEM

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Automated chromatographic method development systems have existed for a number of years. Difficulties in tracking peaks between experiments can limit these systems, especially for samples such as impurities and degradants which may have many trace components to resolve. Automated method development systems typically lack versatility, meaning that established method development routines must be adjusted in order to fit the capabilities of the automated system. Finally, for complex problems, method development may involve many experiments with many samples (including composite samples), and a large amount of data can be generated. It can take a significant amount of human time and effort to track, review, and manage these data.

This presentation will describe a new system for automated method development which addresses some of the weaknesses of earlier configurations. The tool uses both UV-Vis and MS detection to unequivocally track and resolve trace components, performing a chemometric evaluation of these detection techniques. The system also includes data handling and storage systems designed to quickly summarize the information, and reduce the time required for data review and report creation. A wide array of instrument configurations and method development approaches is supported, making the system flexible for many types of laboratories.

SIMULTANEOUS DETERMINATION AND SPECIATION OF AS AND SE IN URINE AFTER INGESTION OF SEAFOOD WITH OR WITHOUT SELENIUM SUPPLEMENT BY HPLC/ICPMS

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A robust method for the simultaneous determination of six Se species and six As species in human urine by reversed-phase liquid chromatography with ion-pair reagent coupled with ICP-MS has been developed. *Selenium* species, trimethylselenonium ion (TMSe), selenocystine (SeCys), selenite (Se(IV)), selenourea (SeUr), selenomethionine (SeMet), selenoethionine (SeEt), and arsenic species, arsenocholine (AsC), arsenobetaine (AsB), dimethylarsinic acid (DMA), methylarsonic acid (MMA), arsenite (AsIII), and arsenate (AsV) were separated on a Waters Symmetry C8 reversed-phase column by eluting with tetramethylammonium hydroxide at pH 5.7-5.8 adjusted by malonic acid. The total analysis took less than 10 minutes with isocratic elution at a flow rate of 1.0 ml/min and was free from the chloride isotope interference due to the complete separation of arsenic species from chloride. The estimated detection limits in synthetic urine at injection volume of 50 μ L ranged between 0.10 and 0.43 ng/mL for As species and between 0.71 and 1.53 ng/mL for Se species.

This method was applied to study the relationship between urinary As and Se metabolites after ingestion of 1-pound seafood with or without 1.0 mg of *selenium* supplement (as high *selenium* yeast) by one volunteer. From total analysis, As excretion increased by 31.4% with the presence of *selenium* supplement in the ingestion; Se excretion increased by 28.2% with the presence of seafood in the ingestion. The excretion of AsB and MMA increased about 100% with the ingestion of L-selenomethionine, while the DMA excretion changed a little. From the time resolving distribution of As and Se species, it was observed that the excretions of MMA, DMA, TMSe, and selenosugar were delayed, which may be explained by the competition for methyl group between As and Se species. This study suggests that Se intake may be positively associated with urinary As excretion, and may alter As metabolite distribution in humans, and vice versa. More experiments are needed to be carried out to confirm these results.

SPECIATION OF SELENIUM COMPOUNDS BY CAPILLARY ELECTROCHROMATOGRAPHY INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY

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A T-type interface and a cross-flow nebulizer were introduced to find ways to combine capillary electrochromatog-

raphy (CEC) with inductively coupled plasma mass spectrometry (ICP-MS) detection for analysis of *selenium* speciation. For the CEC separation, we employed a macrocyclic polyamine bonded phase capillary as the separation column and a bare fused silica filled with the make-up liquid. To optimize the measurement, we investigated several parameters: nebulizer gas flow rate; rotation rate of peristaltic pump; type, concentration, and pH of the mobile phase; and applied voltage. With Tris buffer (pH 8.5, 50 mM), applied voltage of -15 kV, ICP Rf power of 1200 W, nebulizer gas flow rate of 1.02 l/min, and lens voltage of 6 V, the reproducibility for the retention time indicated that sample injected by electrokinetic and nebulizer gas flow (RSD < 1.38%) was better than that by self-aspiration alone (RSD < 1.79%). The detection limits for selenate, selenite, selenocystine, and selenomethionine were found to be 2.40, 3.53, 12.86, and 11.25 ng/mL, respectively. Due to the high sensitivity and element-specific detection, as well as the high selectivity of the bonded phase, quantitative analysis of *selenium* speciation in urine was also achieved.

THEORETICAL STUDIES OF ONLINE IMMUNOEXTRACTION COUPLED WITH REVERSED-PHASE LIQUID CHROMATOGRAPHY

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Online immunoextraction (IE) coupled with reversed-phase liquid chromatography (RPLC) is an example of a multidimensional technique that can be used to separate and analyze structurally similar compounds from complex sample mixtures. IE makes use of the strong and selective binding of antibodies to remove and concentrate a certain group of compounds from a sample. RPLC then allows the separation of structurally similar chemicals that bind to the same IE column. IE/RPLC usually involves three columns. The first column performs immunoextraction. As analytes elute from the antibody column, a small, reversed-phase precolumn is used to trap and concentrate them before they enter the final column, the RPLC analytical column. An important component of a coupled IE/RPLC system is the interface between the IE column and the precolumn. However, little information is available on the behavior of analytes at this interface. In an effort to better understand this aspect of IE/RPLC, well-characterized analytes were used as models. The compounds studied included various chlorophenoxyacetic acid herbicides. The elution of such compounds from an IE/RPLC system under different conditions was predicted and optimized using chromatographic theory along with the known binding and dissociation rates of the IE columns and retention of the analytes on RPLC phases. Good agreement was noted between the predicted and experimental results.

ACKNOWLEDGMENT

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METHOD DEVELOPMENT IN PHASE OPTIMIZED LIQUID CHROMATOGRAPHY

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Several strategies for method development in HPLC are used at present. Some scientists remain the stationary phase constant and optimize the mobile phase composition (pH, nature of mobile phase, buffer concentration) and the temperature.

Recent publications [1] recommend, in addition, a variation of the stationary phase, whereas stationary phases with selectivity as different ("orthogonal") as possible should be used.

In phase optimized liquid chromatography (POPLC) the optimization of the stationary phase is the first goal. Due to the fact that retention times are additive, different stationary phase segments are coupled together to create the column with the best overall performance for each particular separation. The retention time of each analyte is controlled by the use of the best selective column for this purpose. In POPLC, the optimum column has to be found in an isocratic way. Nevertheless, if the mixture is very complex and covers a wide range of polarity, gradient elution cannot be overcome.

In this paper, we show how to optimize a separation for an unknown very complex mixture from chemical industry. The sample is completely unknown and no standards are available. It is demonstrated how we apply the concept of POPLC and which strategy has to be applied to get this method development done.

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MONITORING METABOLISM OF *ESCHERICHIA COLI* USING CAPILLARY ELECTROPHORESIS AND CAPILLARY ELECTROPHORESIS MASS SPECTROMETRY

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Escherichia coli (*E. coli*) is perhaps the most studied of all model organisms. Pathogenic strains, such as *E. coli* O157 : H7, pose a significant risk to human health. Recent findings using whole-genome expression profiling revealed that *E. coli* MG1655 genes were induced by the nutrients available in the mammalian intestine. The mutational analysis/microarray study found that only mutations in sugar pathways affected

the colonization of the bacteria. The goal of this study was to determine the in vitro metabolism preference of two different colonizing *E. coli* strains (MG1655 and EDL933) towards the 14 monosaccharides known to be found in intestinal mucus. Carbohydrate concentrations were monitored using capillary electrophoresis (CE) with different detection methodologies. The disappearance of each sugar in the growth media provided the nutritional preference of *E. coli*. No single capillary electrophoresis method could be developed to monitor all the carbohydrates simultaneously. Instead, three independent CE methods were required. The combination of CE-UV and CE-MS data provided a complete picture of the actual carbohydrate metabolism of *E. coli* from colonization to constant phase. The data showed that different strains have different nutritional preferences. MG1655 did not metabolize N-acetylgalactosamine, while EDL933 did. Other sugars changed in their order of metabolism. The in vitro model clearly indicates that certain sugars are metabolized only during colonization and others during maintenance. This in vitro model provided an enhanced view of the actual carbohydrate preference of two different strains of *E. coli* and demonstrated significant differences between the commensal and pathogenic strains.

APPLICATION OF ONLINE RAPID SAMPLING MICRODIALYSIS TO CLINICAL MONITORING OF PATIENTS DURING GASTROINTESTINAL SURGERY

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We have previously developed a rapid sampling online microdialysis technique for monitoring metabolites in the injured human brain [1]. More recently, we have adapted the system to monitor the brain during surgery and this proved to have advantages in providing a metabolic feedback to the surgeon interoperatively [2]. We are now seeking to develop an analytical system that allows to use the rapid sampling microdialysis during bowel and stomach surgeries.

Gastrointestinal surgery is one of the most common surgical procedures in the developed world. Complications associated with this type of surgery can be severe enough to lead to mortality [3]. Intestinal ischemia during and after surgery is a major factor that can lead to multiple organ failure [4]. Monitoring metabolic changes in the digestive system during surgery is of great interest as an early marker of intestinal ischemia. Previous attempts to monitor the bowel had monitored intraperitoneally in experimental models [4]. Whilst some evidence of ischemia was found, the poor time resolution of the assay coupled to the large volume of the intraperitoneal cavity prevented this from being useful during human bowel surgery. We have, for the first time, implanted a microdialysis probe into the gut wall. Optimization of the online assay for bowel uses has allowed us to observe the metabolic condition of the tissue online throughout the

surgery. This also has potential applications postoperatively to detect anastomosis leak.

The online monitoring system electrochemically analyzes the dialysate for glucose and lactate at high time resolution (typically 30-second sampling). The method couples flow injection analysis to enzyme-based amperometry [1].

We will describe preliminary results from monitoring patients in St. Mary's Hospital, Imperial College, with this technique.

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SUBSECOND FFT CONTINUOUS STRIPPING CYCLIC VOLTAMMETRIC TECHNIQUE AS A NOVEL METHOD FOR PICO-LEVEL MONITORING OF IMIPRAMINE AT AU MICROELECTRODE IN FLOWING SOLUTIONS

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A novel method for the determination of imipramine in flow-injection systems has been developed. The method was used for the fast determination of imipramine in its pharmaceutical formulations and biological samples. The developed technique is very simple, precise, accurate, time-saving, and economic as compared to all previously reported methods. The effects of various parameters on the sensitivity of the method were investigated. The best performance was obtained with the conditions, pH value of 2.0, sweep rate value of 60 V/s, accumulation potential of 100 mV, and accumulation time of 0.5 second. In this work, we introduce a special computer-based numerical method, for calculation of the analyte signal and noise reduction. The electrode response was calculated based on partial and total charge exchanges at the electrode surface after subtracting the background current from noise. The waveform potential consisting of potential steps for cleaning and accumulation of analyte, and potential ramp were applied on an Au disk microelectrode (with a radius of 12.5 μm). The method was linear over the concentration range of 25-75000 $\mu\text{g/mL}$ ($r = 0.997$) with a

limits of detection and quantitation of 13 and 30 $\mu\text{g/mL}$, respectively. The method has the requisite accuracy, sensitivity, precision, and selectivity to assay imipramine in tablet and plasma.

THE NUMEROUS SAMPLE CONDITIONING AND SAMPLE PREPARATION OPTIONS OFFERED BY AN AT-LINE ANALYSIS SYSTEM

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Sample conditioning and sample preparation represent time-consuming activities involving many steps that have to be performed manually. At high sample throughputs, these routine tasks constitute a considerable cost factor. Generally, analytical systems that carry out these steps automatically have a low-payback time due to lower costs, lower contamination risks, and thus more reliable analytical results. Various sample conditioning and sample preparation techniques have already been put into practice using the ProcessLab system to be described:

- (i) filtration of water samples;
- (ii) extraction of organic substances;
- (iii) derivatization of analytes;
- (iv) digestion by heating under reflux;
- (v) elimination of interfering components.

This paper presents a detailed description of some already realized solutions.

Filtration of water samples

Water samples for ion chromatographic (IC) analysis must be free of solid particles as these can block the capillary columns. Polluted water samples must therefore be filtrated prior to chromatographic separation. After dilution with eluent, the sample aliquot is ultra-filtrated and subsequently transferred to the IC system. After the detection of the ionic ingredients of the sample, the system automatically starts the filtration of the next water sample.

Extraction of organic substances

The setup for the automatic extraction of some organic substances from water samples is described. In this case, a liquid-liquid extraction is used to extract organic compounds from the aqueous sample using an *n*-alkane. The organic phase is used for further spectroscopic and gas chromatographic determinations.

Digestion by heating under reflux

With the available digestion module, it is possible to digest a considerable number of components prior to further analysis. In this example, the automatic determination of

total phosphate and orthophosphate in water samples is described.

The modular ProcessLab system offers several sample preparation options and thus can be adapted to a large variety of sample types. The automation of different preparation steps enhances sample throughput and repeatability of the results. After sample preparation, either the analysis is performed in the module or samples are transferred to external analytical systems such as chromatographic or spectroscopic devices.

The highly flexible ProcessLab adapts to many analytical challenges.

UNATTENDED INLINE AND SEQUENTIAL SAMPLE CONCENTRATION FOR A RANGE OF APPLICATIONS

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Many techniques are used for the concentration of samples and the level of labor, user supervision, and recoveries vary with each labor. This unattended concentration technique allows the operator to concentrate samples from an inline process such as GPC cleanup and solid phase extraction (SPE) or concentrate precollected samples ranging in volumes from a few to several hundred milliliters. The samples are sequentially evaporated under microprocessor control with sensor feedback, concentrated to a final endpoint with solvent exchange, and transferred to a storage vial or GC vial ready for analysis.

Recoveries are demonstrated for a range of applications from environment, food, and pharmaceuticals. These reduced the exposure to hazardous solvents and other chemicals.

QUALITY CONTROL OF FRENCH FUEL BY NIR CHEMOMETRIC ANALYSIS

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The aim of this study was to demonstrate that near infrared (NIR) method combined with multivariate analysis allows the classification of the different commercial fuels (gasoline and diesel) and highlights their large quality variability.

Even though fuels have to reach commercial quality specifications, complexity of refining tools and crude oils slates involves large variability in terms of finished products qualities.

The study was carried out in south east of France, on an area covering about 20 squared miles where four major oil companies are located.

20 gasolines and 15 diesel have been collected, taken from different brand gas stations and the supermarkets (Figure 5). Many fuel mixtures have been prepared, 160 with



FIGURE 5: Sample location area.

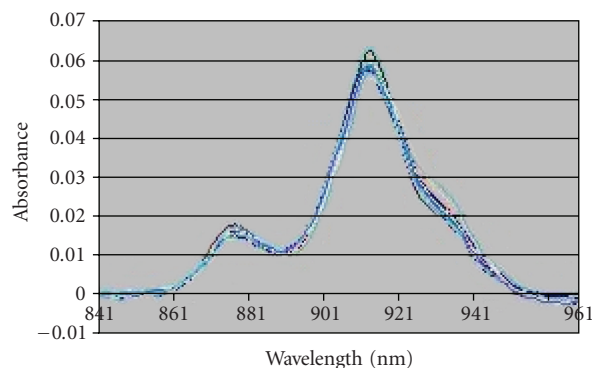


FIGURE 6: Commercial gasolines NIR spectra.

different gasolines and 24 with different diesel. Conventional properties (RON, MON, density, cetane number, etc.) were measured and the NIR spectrum was collected for each sample.

FT-NIR spectra were recorded with a Nicolet Antaris spectrometer interfaced to a personal computer. Diesel samples were filled into a 4 mm path length borosilicate glass tube. All the spectra were computed at 4 cm^{-1} resolution between 4500 and 10000 cm^{-1} —thanks to the software result integration 2.1 Thermo Nicolet. Coaddition of symmetrical interferograms on 10 scans was performed for each spectrum. A reference spectrum was recorded before each sample measurement on an empty tube. Gasoline spectra were collected—thanks to a Hellma probe with immersion, connected to the same spectrometer by NIR optical fibers.

It is impossible to differentiate one gasoline from another (Figure 6), or one diesel from another (Figure 7), just looking at spectra, since profiles are very similar. Thus, principal component analysis (PCA) was performed on the near infrared datasets to classify and distribute the products across specifically defined groups. Those groups are in agreement with the measured properties of the fuels. Partial least-squares method (PLS) was used to model the conventional properties of gasolines and diesel.

This study highlighted that the variability of fuel quality can be approached by near infrared techniques combined with statistical analysis.

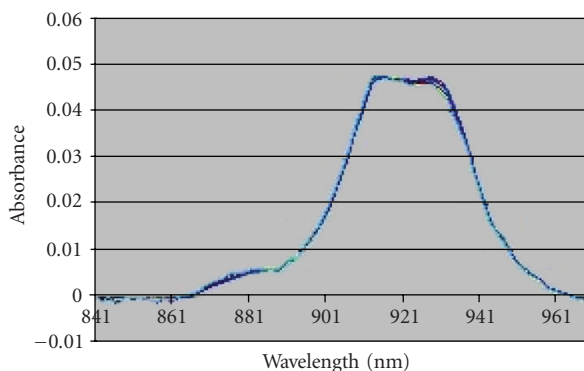


FIGURE 7: Commercial diesel NIR spectra.

AUTOMATED MERCURY ANALYSIS IN LIGHT AND HEAVY HYDROCARBONS

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Naturally occurring mercury in hydrocarbon matrices is very problematic for the hydrocarbon processing industry. It is known to poison the catalysts used in refining and chemical production, as well as causing corrosion in aluminum heat exchangers. These detrimental impacts of mercury are very costly and hazardous. As a result, it has become increasingly important to monitor the levels of mercury in the hydrocarbon processing industry.

In this paper, a new automated technique for accurately quantitating mercury in both light and heavy hydrocarbons is discussed. This new technique employs automated sampling, pyrolysis, gold amalgamation, and cold vapor atomic absorption spectroscopy (CVAAS).

Supporting data will be provided to illustrate the high-quality results obtained through the use of such an analyzer. The capabilities of this new technique will be discussed in detail.

RAPID AUTOMATED PRESSURIZED SOLVENT EXTRACTION OF CHEMICAL WARFARE AGENTS FROM SOIL SAMPLES

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The rapid extraction of chemical warfare agent surrogate compounds from soil samples was conducted using an automated pressurized solvent extraction instrument. Organophosphorus pesticides were used as surrogate compounds for nerve agents and extracted from soil samples to evaluate the pressurized parallel solvent extraction technology for this application. The results were compared to traditional solvent extraction techniques for recovery and reproducibility.

In addition, the ability to extract and process high-volume sample loads rapidly was evaluated in a working environmental laboratory using the extraction of PCBs from soil as a test model. Data are presented detailing the procedures, manpower, and equipment required to extract 200 soil samples in one day for chemical warfare agents.

THE DEVELOPMENT OF A SEARCHABLE COMMERCIAL PRODUCTS LIBRARY FOR DART SPECTRA

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The DART is one of the greatest advances in technology in our fight against crime. It offers real-time analysis of evidence as it was discovered by law enforcement officers in the field generating exact mass measurements and patterns which are then used for identification. The DART system is composed of an ionization source that uses helium in its excited state to ionize a sample that is placed in the gas stream and an accurate mass time-of-flight mass spectrometer to detect the ions produced. This direct analysis in real-time (DART) method of ionization is most similar to electrospray ionization and usually, but not always, generates M+1 or M-1 ions. But unlike electrospray, analysis by DART generates a pattern instead of a single peak because there is no separation of a sample into its individual components—like with gas chromatography or liquid chromatography—prior to entering the mass detector. The DART is essentially generating a unique picture (mass spectrum) of numbers (exact masses) and lines (patterns) to represent the components in a specific sample, like the ingredients in a commercial product. For example, the analysis of two different soft drinks would generate two very unique pattern-specific mass spectra that could be used to narrow the identification of an unknown brown liquid received by the laboratory as having ingredients consistent with a soda, but they would also differentiate between the two and positively identify them as Brand X. As this technology is new, however, there are no databases or libraries in existence to search DART spectra against to identify commercial products. The FBI is currently working to develop and populate a searchable commercial products library.

AN AUTOMATED GC/MS LIBRARY SCREENING METHOD FOR FORENSIC SAMPLES

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In most forensic analyses, a variety of analytical strategies are implemented for unknown cases from general screening methodologies for classes of drugs to specific confirmation and quantitation of individual drugs. Despite the improvements in instrument analysis time, two areas of sample analysis have been lagging: (1) sample preparation and (2) data

processing. In this study, the focus is on the data processing of samples acquired from an acid/neutral screening method utilizing a gas chromatograph/mass spectrometer.

Currently, interrogating an unknown sample (entire data file) against a library is still a manual process and requires enormous amount of time. As an alternative to common library search techniques, this study will demonstrate an automated analysis method combined with the use of a mass spectral deconvolution software that rapidly screens the entire GC/MS data for a targeted list of compounds in the library and provides both qualitative and quantitative information. In some cases, just knowing that a drug is present is all that is needed. However, in other toxicological analyses, knowing how much of a drug is present is critical.

In this study, the database of acid/neutral drugs is generated and used to screen blind samples, as a way to simulate unknown sample, against the database for both qualitative and quantitative investigations. The results demonstrate that it is possible to use deconvolution software as a tool for interrogating unknown samples. As to pursue the current trend in expediting sample analysis time, data will show that it is possible to reduce the overall analysis time and yet maintain data quality.

IDENTIFICATION OF PRECURSOR, INTERMEDIATE, AND FINAL PRODUCTS ROUTINELY SEIZED FROM CLANDESTINE DRUG LABS BY FTIR-ATR SPECTROSCOPY

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While methamphetamine and many other illicit drugs of abuse do not occur naturally, they are synthesized in large quantities in clandestine laboratories across the country. Unfortunately, the materials (e.g., precursors, reagents, and solvents) are commercially available and recipes are easily uncovered on the internet. The role of the forensic chemist is to rapidly screen and identify a large number of seized evidence samples. Previous research has shown the spectroscopic identification of these types of materials to be attractive due to the inherent capabilities of real-time identification of volatile solvents, nondestructive analysis which allows analysis by a primary identification technique, and minimal sample preparation. This work will show the advantages of using FTIR-ATR spectroscopy for the forensic screening of unknown confiscated materials from suspected clandestine drug labs.

IDENTIFICATION OF THE VOLATILE ORGANIC COMPOUNDS PRESENT IN DECAYING PIG ORGANS USING SPME-GC/MS

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Identification of the volatile organic compounds (VOCs) that result from human decomposition have been studied

by many law enforcement groups in an effort to improve cadaver dog training procedures and to detect human remains in clandestine graves. Although studies of VOCs from whole pig decomposition have been published, there is little information about the compounds that result from the decomposition of individual pig organs. Solid phase microextraction (SPME) coupled with gas chromatography mass spectrometry (GC/MS) was used to analyze the headspace of the decomposing pig tissues over a period of three months. Analyses of tissue samples from the decomposition of pig skin, muscle, blood, heart, liver, intestine, lung, fat, and bone were monitored every few hours for the first couple of days, then weekly for a period of two months. This paper will report on the VOCs identified from pig tissue samples and compare them to the VOCs that have been identified from human decomposition.

DETECTION OF NITRITE BY FLOW INJECTION ANALYSIS USING A NOVEL PAIRED EMITTER-DETECTOR DIODE (PEDD) AS A PHOTOMETRIC DETECTOR

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An inexpensive flow injection system for determining low concentration levels of nitrite employing the greiss reagent spectrophotometric method is reported. In most microanalytical systems, neither the light source nor the photodetector is integrated into the same substrate as the fluidic channel network. This is because the integration of all components necessary for performing a total chemical analysis is very complex [1]. The work presented herein focuses on a novel integrated optical sensor, the PEDD.

The novel flow detector applied within this manifold is a highly sensitive, low-cost, miniaturized light emitting diode (LED-) based flow detector. This colorimetric detector employs two LEDs, operating one as a light source and the other as a light detector. The emitter LED is forward-biased and the detector is reverse-biased. The emitter and detector LEDs had a λ_{max} of 530 nm and 660 nm, respectively. The emission spectrum of the emitter LED efficiently overlapped with the absorbance spectrum of $9 \mu\text{M}$ NO_2 as shown in Figure 8. A simple timer circuit measures the time taken for the photocurrent generated by the emitter LED to discharge the detector LED from 5 V (logic 1) to 1.7 V (logic 0) [2].

The optimum emitter LED light intensity was investigated as decreasing light intensity had previously proven to increase the response obtained [3]. The flow rate, dynamic range, sensitivity, and limits of detection were investigated. Detection limits in the nanomolar range were achieved using the PEDD. For a comparative study, the dynamic range and limits of detection were also investigated using a plate-well reader. Higher sensitivity and improved precision were obtained from the PEDD compared to the commercially available plate-well reader.

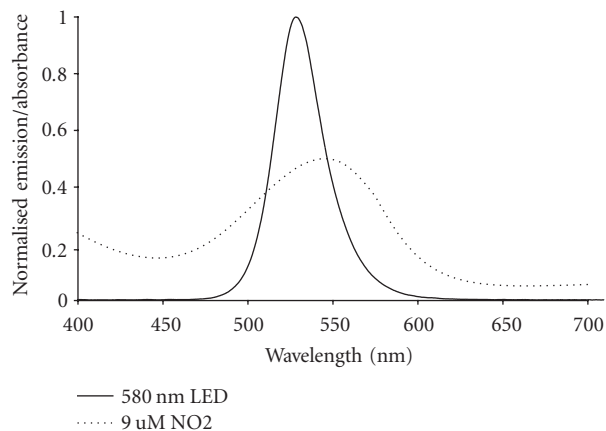


FIGURE 8

ACKNOWLEDGMENTS

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AUTOMATED, HIGH-THROUGHPUT AMMONIA MEASUREMENT USING AN IMPROVED GAS-SENSING ELECTRODE EQUIPPED WITH AN AUTOSAMPLER

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Automated high-throughput ammonia measurement is considered to be highly desirable in the field of water management. The simplicity of ISE-based ammonia gas-sensing electrodes has made them the workhorses for ammonia measurements in laboratories and in fields of environmental monitoring. However, the success of automation was often hampered by the electrodes' inadequate response rate and recovery time, especially in samples with ammonia at levels lower than 1 ppm, leading to unsatisfactory accuracy and repeatability. The high impedance of inner pH glass membrane also makes the electrode more susceptible to the cable movement using the autosampler during the measurement.

This paper describes a newly developed system that integrates an improved ammonia gas-sensing electrode with a meter-controlled autosampler for automated, high-throughput ammonia measurements. The new electrode not only incorporates a glass membrane of lower resistance to reduce the susceptibility of the sensor response to the cable movement using the autosampler, but also has an optimized configuration to improve the response rate, accuracy, and repeatability. In addition, the new electrode acquires ease-of-handling features such as a translucent body with a fill line mark, a back seal, and a preassembled membrane cap. Method development using the new ammonia gas-sensing electrode with the autosampler is discussed. Different ISE analysis methods, such as direct measurement, known addition, and Gran method, are compared. Factors affecting the measurement are studied, optimum is set up, and analytical

sequences for various samples are determined. Results show that the analytical process of ammonia measurement including electrode rinsing and calibration, reagent/standard addition, and sample measurement can be carried out automatically without being attended by an operator, per sample analysis may take less than 3 minutes, and that satisfactory results can be obtained in low-concentration range.

MONITORING WATER-QUALITY PARAMETERS REMOTELY VIA WIRELESS SYSTEMS

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We have designed a low-power water-quality array for four sensors (pH, conductivity, temperature, and dissolved oxygen) that can be interfaced to a radiofrequency (RF) wireless mote. RF "motes" are low-power devices that incorporate sensors, processing, and communications in a small package, which were developed by the University of California at Berkeley in 1999.

The RF motes can receive data from multiple sensors and communicate with each other to transmit data to a remotely located PC. In particular, we discuss remote monitoring of local bodies of water surrounding the Suffolk University environmental research facility at Cobscook Bay in Edmunds township, Maine. The water-quality sensors are interfaced to a field mote which sends data to the base mote connected by USB to a dynamic web server at the research field station. The monitoring allows year-round study of an area with 18 ft tidal fluctuations and harsh winters. The data are archived for analysis in a database and displayed in "real time" at Suffolk University.

We will describe techniques used to overcome intermote distance limitations, interface issues of the sensor board to the motes, solutions to long term power for the motes in the field, effects of submerging the sensor/mote package in water, remote calibration of the sensors, quality of the data obtained, and software modifications that are necessary for operation.

AUTOMATED ANALYSIS OF AQUEOUS SAMPLES CONTAINING PESTICIDES, ACIDIC/BASIC/NEUTRAL SEMIVOLATILES, AND VOLATILE ORGANIC COMPOUNDS BY SOLID PHASE EXTRACTION COUPLED INLINE TO LARGE VOLUME INJECTION GC/MS

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Data are presented on the development of a new automated system combining solid phase extraction (SPE) with GC/MS spectrometry for the single-run analysis of water samples

containing a broad range of organic compounds. The system uses commercially available automated inline 10 mL sample extraction with large volume injection and GC/MS. Two commercially available SPE sorbents used in series allow extraction of both polar and nonpolar compounds. Inline injection of the 90 μ L extract from a 10 mL sample resulted in detection limits 10–100 times lower than recommended EPA method 8270 limits for semi-volatiles. Variance in SPE extraction efficiency is directly monitored and quantitative accuracy improved through the use of “internal” deuterated and nondeuterated standards/surrogates added to calibration standards, blanks and samples prior to automated SPE concentration. System performance has been demonstrated for 92 target organic compounds, including acidic, basic and neutral semivolatile compounds, as well as chlorinated- and nitrogen-containing pesticides. This detection system has also been successfully applied to the analysis of a range of Method 8270 semivolatile compounds in complex matrices derived from water-isopropanol extracts of contaminated sediments, resulting in enhanced sample throughput with optimum detection limits. Preliminary work has also demonstrated the potential for quantitation of both volatile and semivolatile compounds in a single analysis. The performance and flexibility of this system along with increases in automation and reduction in solvent usage and accompanying analyst exposure make this an attractive alternative for the analysis of volatile and semivolatile organic compounds in aqueous samples.

ACKNOWLEDGMENT

The work is funded by the USEPA.

CHIRAL SCREENING USING A SINGLE ZIRCONIA COLUMN AND MULTIPLE BONDED CHIRAL STATIONARY PHASES

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We report here the synthesis and testing of a novel polysaccharide-class chiral HPLC stationary phases based on porous zirconia. A new method for the attachment of polysaccharide with anchor groups to zirconia surface through Lewis acid-base attachment chemistry is described. Generally, the attachment of a specific polysaccharide chiral selector (CS), which contains multiple phosphonic acid anchor groups per polymer chain that can then be further bonded to a zirconia surface through a Lewis acid-base reaction, is shown. Furthermore, the synthesis of a variety of brush-type chiral selectors that contain anchor groups for zirconia that can be easily attached to/removed from CSPs for chiral selector screening is described. The optimum chiral separation conditions including mobile phase compositions and mobile phase additives were studied. The selectivity and efficiency of the new zirconia-based phases to analogous silica-based chiral stationary phases are compared. The

results indicate that the two substrates can perform similar enantiomer separations, but there are differences in the selectivity for most analytes tested. A number of pharmaceutical chiral separations are shown. The zirconia-based chiral stationary phases were found to be chemically stable from pH 1 to 8 under reversed-phase conditions. We also demonstrate that zirconia columns can be used for doing fast screening of CSPs without the need for using multiple columns.

DETECTION LIMIT AND REACTION EFFICIENCY IMPROVEMENTS IN THE AUTOMATED DETERMINATION OF IONS IN ENVIRONMENTAL SAMPLES USING INJECTION SEGMENTED FLOW ANALYSIS

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Segmented flow analysis (SFA) and flow injection analysis (FIA) are both continuous flow techniques that have been used for automating wet chemical methods. In addition, the flow injection technique has been widely used as a means for rapid and precise sample introduction into a variety of analytical instruments such as atomic absorption and mass spectrometers. However, sample injection has not been previously coupled with segmented flow analysis which is the subject of this paper.

Mixing in FIA depends in part on longitudinal dispersion which also dilutes the sample and decreases sensitivity for long reaction times. In contrast, SFA limits longitudinal dispersion and facilitates longer reaction times, but the standard SFA sampling technique of aspiration is not precise and requires the reaction to be brought to completion. By injecting the sample into a carrier stream which is then segmented with air, longer, but nonsteady state, reaction times can be achieved which combine to increase both sensitivity and sample throughput.

MONITORING OF A COMPLETE PROCESS LINE FOR THE PHOSPHATIZING OF METAL SURFACES USING AN AT-LINE ANALYSIS SYSTEM

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With an estimated worldwide turnover of more than 500 million US dollars, phosphatizing is the most important metal pretreatment process. The phosphatizing process produces a hard, electrically nonconducting surface coating that adheres tightly to the underlying metal. This layer protects the metal from corrosion and improves the adhesion of paints and organic finishes to be subsequently applied.

The basic phosphatizing process consists of the etching reaction and the formation of the surface coating. After thoroughly degreasing and rinsing the metal workpieces, the phosphoric acid removes interfering surface-bound metal

oxides and increases the surface roughness. Subsequently, the alkali phosphates react with the previously generated metal ions forming a layer of insoluble tertiary metal phosphates. Addition of metal cations such as Zn^{2+} , Mn^{2+} , Ca^{2+} , or Ni^{2+} to the phosphatizing bath improves the quality of the coating.

At the industrial scale, the phosphate coatings are applied to the metal surface of the workpieces using strictly defined process steps in different cleaning, degreasing, rinsing, activation, and phosphatizing baths. The various bath parameters have to be closely monitored as they determine to a large extent the quality of the coating produced. The parameters determined in the cleaning, degreasing, and rinsing baths are pH value, conductivity, plus free and total alkalinity, while the phosphatizing bath is analyzed for free and total acids, nitrite or hydrogen peroxide, and zinc and fluoride.

The described ProcessLab at-line analysis system controls, records, and documents the important analytical parameters of the entire phosphatizing process. The combination of the analytical methods involved as well as the straightforward and error-free handling via the well-organized user interface allows for complete process control. The analytical functions are supplemented by the integrated operation software that offers numerous possibilities for data processing and documentation of the measured values. Additionally, a bar code reader guarantees an unambiguous sample identification.

The at-line analysis system meets all requirements of modern phosphatizing plants regarding process monitoring and documentation.

COMPREHENSIVE AND FULLY AUTOMATED WATER ANALYSIS

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Water contamination is a widespread environmental problem. Every year, some 700 million tons of waste enter the aqueous systems and thus reduce our already limited freshwater resources. Maximum contaminant levels (MCLs) in drinking water are specified for a large number of inorganic ions in order to minimize potential health hazards. Highly effective monitoring tools are required for assessing the occurrence, the fate, and the environmental impact of contaminants.

The analysis system described here combines titration, ion chromatography, and direct measurement. It has been specially developed for automated drinking water analysis but can be adapted to suit any number of analytical requirements in the food, electroplating, or pharmaceutical industries. The emphasis has been placed on user-friendly operation, variable sample container volumes, and protection of the samples from ambient interferences. The standard version measures the following sample parameters fully automatically and simultaneously: temperature, pH, and conductivity; it titrates acidity, calcium, and magnesium and deter-

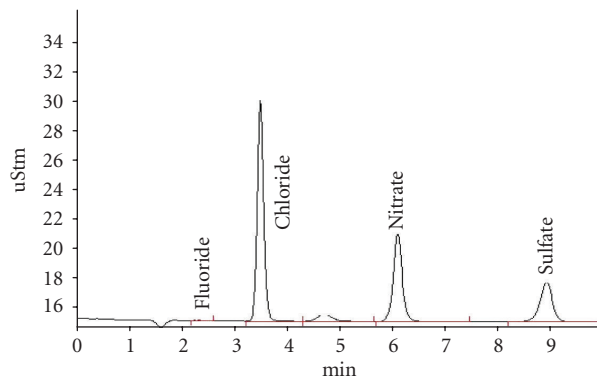


FIGURE 9: Ion chromatogram of tap water from Herisau (column: Metrosep A Supp 5 – 100; eluent: 3.2 mmol/L Na_2CO_3 /1.0 mmol/L $NaHCO_3$; flow: 0.7 mL/min).

mines anions with ion chromatography (Figure 9). All results are obtained within nine minutes. The comfortable and user-friendly software stores and manages the results in a database.

The system's flexibility allows implementing additional measurements extending the range of ions to be determined. The system equipped with two ion chromatographs allows for a simultaneous determination of all cations and anions. By calculating the difference between positive and negative total charges, the ion balance can be determined. Besides the integration of various sample preparation techniques, such as dilution, fully automated ultrafiltration, or dialysis, it is possible to automatically remove the lids from sealed sample vessels prior to analysis.

The authors will give a detailed description of the setup and present results obtained by analyzing tap water from Herisau (Switzerland).

SIMULTANEOUS PRECONCENTRATION AND DETERMINATION OF COPPER, NICKEL, COBALT, AND LEAD IONS CONTENTS BY FLAME ATOMIC ABSORPTION SPECTROMETRY

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A sensitive and simple method for the simultaneous preconcentration of nutritionally important minerals in real samples has been reported. The method is based on the formation of mineral complexes by *n*-4-propyl-2-thiouracil (PUT) supported on activated carbon. The metals contents on the complexes are then eluted using 5 mL 6 M HNO_3 , which are detected by AAS at respective maximum wavelengths. In this procedure, minerals such as Cu, Ni, Pb, and Co could be analyzed in one run by carrying out the simultaneous separation and quantification of them. The low detection limits of these elements in this technique make it a superior alternative to UV-Vis and in several applications, also an alternative to ICP-MS techniques. The method has been successfully applied

for these metals content evaluation in some real samples including waste water, river water, spring water, tap water, vegetable, and cow liver.

DETERMINATION OF WATER IN FOOD BY AUTOMATED TITRATION

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Karl Fischer titration is a method for determining the water content in different matrices, based on a selective reaction between water and the Karl Fischer reagent. In comparison to traditional drying methods, the Karl Fischer titration is less time-consuming and often more accurate; drying methods quantify the mass loss, which can differ from the water content, especially in foodstuffs.

Low water contents from approximately 0.001% to 1% can be detected by coulometric Karl Fischer titration; here the iodine required is generated electrochemically in the titration vessel. Higher water contents from approximately 1% up to 100% can be determined by volumetric Karl Fischer titration, where an iodine solution serves as titrant.

Automated Karl Fischer titration allows to deal with more samples in less time; automated sequences can include different methods for the determination of titers and blank values and for different types of samples. Moreover, automation generally improves reproducibility and accuracy.

In this work, standard Karl Fischer methods using commercially available water standards and solvents were developed using an automated Karl Fischer titration system. Both volumetric and coulometric techniques were applied to different edible oils such as soy bean, sun flower, olive, rapeseed, sesame, and pumpkin seed oil.

An essential prerequisite for automated Karl Fischer titration is complete dissolution of the sample or at least a suspension or emulsion as homogeneous as possible, as the samples are transferred from sealed glass vials through a transfer tube to the titration vessel.

THE HIGH-THROUGHPUT PRESSURIZED SOLVENT EXTRACTION OF PESTICIDE RESIDUE FROM FOODS

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The traditional methods used to prepare food samples for pesticide residue analysis consume large quantities of solvents and large amounts of laboratory time in the sample workup process.

This paper describes the application of pressurized solvents in the simultaneous extraction of six samples for the

analysis of organochlorine pesticide residues. The results are compared to the traditional solvent extraction technique. Many different food matrices (vegetable and grains) are analyzed and the preparation steps for the successful processing of food samples are detailed.

In addition, the procedures, equipment, and manpower required to process a large number of samples in an 8-hour operating day are described.

EVALUATION OF LVI-GC-MS IN PESTICIDE MULTIRESIDUES ANALYSIS USING A PTV INJECTOR WITH AUTOMATED LINER EXCHANGE

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PTV injector with automated liner exchange was used for large volume injection (LVI; up to 200 μ L) in GC-MS analysis of pesticide multiresidues with the aim to obtain high sensitivity and/or simplify sample preparation procedures, for example, evaporation and solvent exchange steps. The system performance, for example, recovery, linearity, and repeatability, was evaluated for 106 pesticides—including organochlorine, carbamate, organophosphorous, pyrethroid and other pesticides—dissolved in acetonitrile and acetonitrile/toluene (3/1) mixture with analyte protectant, for example, L-Gulonic acid \bullet -lactone. Liner exchange period was also evaluated for the 106 pesticides fortified in the extracts of cucumber and spinach obtained from supercritical fluid extraction (SFE).

NOVEL SEMIAUTOMATIC APPROACH FOR PREPARATION AND ANALYSIS OF DRUGS IN LIMITED BIOLOGICAL SAMPLES

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In this paper, we will present

- (1) a rapid, sensitive, and simple LCMS method for the analysis of paclitaxel and its metabolites in biological samples such as arteries, liver, and blood;
- (2) the method will also describe a novel semiautomatic machine used in the preparation of small amount of biological samples for the analysis of drugs at trace level. This machine will be applicable for tissues hard to homogenize such as arteries, ureter, and muscles. This machine increases sample output analysis tremendously as it is capable of loading several samples at a time and provides a more homogeneous preparation than the commercially available manual grinding vessels commonly used for the preparation of limited sample amounts. The homogenizing multiple grinding vessel machine is being patented.

- (3) the method will also discuss the presence of a ghost peak generated from newly purchased Waters UPLC connected to a premier mass spectrometer. The ghost peak interfered with the analysis of paclitaxel and a \$500 K investment for the analysis of paclitaxel to support preclinical studies could have been wasted, had not the ghost peak been successfully eliminated. The ghost peak was UPLC material dependent and was not observed on the micro LCMS system. The technique used to troubleshoot the problem that led to the elimination of the peak and the successful system maintenance will be presented.

GENERAL UNKNOWN SCREENING OF DRUGS AND TOXIC COMPOUNDS IN HUMAN SAMPLES BY MEANS OF A COMPREHENSIVE LC-MS/MS TECHNIQUE BASED ON HYBRID TRIPLE-QUADRUPOLE LINEAR ION-TRAP INSTRUMENTS

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General unknown screening procedures in clinical or forensic toxicology are used to detect and identify the exogenous compounds present in human samples, whether expected or not. A comprehensive GUS method has been developed for drugs, toxic compounds, and their respective metabolites in biological fluids, based on liquid chromatography coupled to a hybrid triple-quadrupole linear ion-trap mass spectrometer.

After nonselective solid phase extraction, the compounds of interest are separated in gradient chromatographic conditions. The mass spectrometer is operated in the information-dependent acquisition mode, where two acquisition conditions are continuously alternated. In the survey scan, enhanced mass spectra are acquired, with dynamic subtraction of background noise in order to detect even tiny peaks in the noise. In the dependent scan, the most intense ions in the survey scan are identified and the instrument instantly switched to the enhanced product ion scan mode to select these ions in the first quadrupole, fragment them in the collision cell using three alternated collision tensions during each scan, and accumulate the resulting fragments in the linear ion-trap to generate rich MS/MS spectra. The complete cycle time lasts for 1.36 seconds. A library of MS/MS spectra of parent compounds and metabolites has been built up and a computer program has been developed to automatically report the results of peak finding and library searching.

This procedure was revealed to be very efficient to identify unexpected compounds in biological samples (as far as they corresponded to library entries), as well as to give clues about the presence of metabolites owing to MS similarities with their respective parent compounds. More than 1000 MS/MS spectra in the positive mode and 250 in the negative mode were entered into the library. Clinical cases will be presented where compounds not found by other screening or target techniques could be identified unambiguously.

96-WELL PROTEIN PRECIPITATION AND ONLINE SOLID PHASE EXTRACTION HPLC TANDEM MASS SPECTROMETRIC METHOD FOR THE DETERMINATION OF ABBOTT DRUG IN DOG PLASMA

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Purpose. To support toxicology studies, a method was developed to determine the concentrations of an Abbott compound in dog plasma. *Method.* HPLC was used to perform online extraction and separation of the compound and internal standard (IS). The compounds were extracted from the dog plasma using 96-well protein precipitation. Hamilton MicroLab was used for the transfer of samples and reagents. A column-switching valve was used to direct the injected sample to the analytical column through the SPE cartridge. A loading/washing pump was used to deliver the loading and wash solution to the SPE cartridge at a flow rate of 2 mL/min and an analytical pump was used to deliver mobile phase to the analytical column at a flow rate of 0.25 mL/min. Applied Biosystems API 4000 MS with Analyst software and ESI interface with positive ionization were used for the analysis. The multiple reaction monitoring (MRM) scans were m/z 349.20/173.10 for the analyte and m/z 354.20/178.10 for IS. *Results.* The quantitative method was shown to be linear over a concentration range of 6.04–3774.98 ng/mL with correlation coefficients (r) greater than 0.99. The evaluation of inter-run assay of QCs was shown to have mean %bias between 2.7% and 4.3% and CV between 3.5% and 4.6%. No interference from blank plasma was observed. *Conclusion.* A fast LC-MS assay for the determination of Abbott compound in dog plasma was validated to determine plasma concentrations following administration of a single dose of drug to Beagle dogs to evaluate the cardiovascular safety profile.

ONLINE COUPLING OF AN IMMOBILIZED TRYPSIN MICROCHIP MONOLITH FOR PROTEIN DIGESTION WITH CAPILLARY MONOLITHIC UHPLC/MS

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Microdevices have proven useful in analytical and clinical chemistry providing much faster analyses, with sensitivity similar to conventional methods. This report describes a monolithic enzymatic microreactor for rapid and complete protein digestion, integrated with a capillary monolithic C18 reversed-phase for peptide separation and ESI-MS for peptide identification. The enzymatic microreactor was prepared through a novel trypsin encapsulation/surface immobilization technique by first encapsulating trypsin in a UV-initiated photo-polymerized monolith consisting of vinyl azlactone, acrylamide, and cross-linker dissolved in

porogenic solvent, then immobilizing additional trypsin on the porous monolith surface through reaction of the azlactone functionality with amine and thiol groups on the enzyme. The enzymatic activity was initially evaluated using on-chip digestion of N-benzoyl-L-arginine ethyl ester (BAEE), insulin chain B, and cytochrome c, and the stability of the enzyme was measured by the change in enzyme activity with time. The optimized microreactor enabled fast digestion of proteins with a low back pressure flow through the microreactor. On-chip digests were directly coupled to a monolithic C18 reversed-phase for peptide separation. The reversed-phase column consisted of a 5 cm long porous poly(stearyl-methacrylate-co-ethylene dimethacrylate) monolith within a 200 μm i.d. capillary, which was coupled with a mass spectrometer for peptide identification. Multiple proteins digested on the enzymatic microreactor- μLC -ESI-MS/MS for system evaluation.

HIGH-SPEED ANALYSIS OF BETA BLOCKERS AND THEIR METABOLITES IN HUMAN PLASMA BY LC/ESI+MS/MS IN HIGH PH MOBILE PHASE

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Beta blockers are important clinical drugs used in the treatment of various cardiovascular disorders. They are toxic and have a very narrow therapeutic range. Beta Blockers are also listed as doping-controlled substances because of their sympathomimetic properties and activity as anabolic agents. It is important to measure and monitor beta blockers in biological samples for disease diagnosing, therapeutic monitoring, doping screening, and forensic analysis.

We developed a high-speed RP LC/ESI+ MS/MS method of analysis for the quantitative determination of nine beta blockers and metabolites acebutolol, alprenolol, atenolol, labetalol, metoprolol, pindolol, propranolol, sotalol, and timolol in human plasma. Beta blockers were extracted from samples using Strata X-C, a mixed mode cation ion exchange, and hydrophilic and hydrophobic SPE sorbents, with simple washing steps. LC separation was performed on a high pH stable Gemini 3 μm C18 50 \times 2.0 mm column, with gradient elution, using 5 mM ammonium bicarbonate, pH = 10, buffer as aqueous mobile phase, and acetonitrile as organic modifier, in 4 minutes. At the same time, LC/ESI+ MS/MS responses of beta blockers were evaluated in 0.1% formic acid in water and acetonitrile mobile phase and compared to responses in high pH mobile phase. Higher signals were recorded in high pH mobile phase than in acidic mobile phase in most cases, and high pH mobile phase provided higher sensitivities for beta blocker analysis. The absolute recoveries of beta blockers from spiked human plasma by SPE were greater than 60% at LOQ of 0.5 ng/mL concentration. Method precision (RSD%) was < 15%, and linear regressions were higher than or equal to 0.999 in a wide dynamic range (>103) while using oxprenolol as internal standard.

HIGH-THROUGHPUT UPLC-ESI-MS MEASUREMENT OF AUXIN

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Background. Although indole-3-acetic acid (IAA), previously named as heteroauxin, is a crucial hormone, a few details of its metabolism and its functions in plants and humans are known, due to the lack of a high-throughput sensitive method measuring IAA and its intermediates/metabolites such as indole-3-carbinol (IOH) and indole-3-acetonitrile (ICN) simultaneously. In the present study, we will discuss IAA measurement using UPLC connected online with either ion-trap or triple-quadrupole MS with ESI interface. **Methods.** The UPLC-ITMS coupling was composed of a Waters Acquity UPLC equipped with a BEH C18 shielded column (1.7 mm, 100 \times 2.1 mm) operated under MassLynx 4.0 software and a Thermo LTQ ITMS operated under Xcalibur 1.4 software. Mobile phases were ammonium acetate and acetonitrile. A Thermo Surveyor HPLC was used to test 6 columns: Synergi hydro-RP18, Onyx monolithic C18, ChromoLith endcapped monolithic RP18, Luna phenyl-hexyl, Luna NH₂, and PolyLC polyhydroxyethyl. The UPLC-MS/MS coupling was composed of an acquity equipped with a BEH C18 shielded column (1.7 mm, 100 \times 1 mm) and a Waters Quattro Premier MS/MS. **Results.** For IAA, IOH, and ICN, the monitored ions were m/z 174 \rightarrow m/z 130 for IAA, m/z 146 \rightarrow m/z 128 for IOH, and m/z 155 for ICN. With conventional HPLC, the PolyLC column was the best in terms of selectivity and sensitivity. Compared to HPLC, UPLC significantly shortened analysis time and increased sensitivity, with the peaks of IAA at 0.74 minute, IOH at 1.28 minutes, and ICN at 2.27 minutes. The UPLC-MS/MS reached IAA LOD to 1 pg per injection (10 μL), much more sensitive than that of 64 pg per injection (5 μL), using the UPLC-ITMS. Using the UPLC-MS/MS, the calibration curves of IAA, IOH, and ICN were linear from 0 to 500 mg/L. **Conclusions.** We have developed a high throughput, sensitive, and accurate UPLC-MS method to determine auxin and its key intermediate metabolites in a 3-minute run analysis.

APPLICATION OF POLYMERIZED MICELLE PSEUDOSTATIONARY PHASE FOR MONITORING OF DOPAMINE BY MICRODIALYSIS AND ONLINE CE-LIF

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In this work, we have investigated conditions for monitoring dopamine in vivo by microdialysis coupled online to electrokinetic chromatography and laser-induced fluorescence (EKC-LIF) using a dendrimer pseudostationary phase (PSP). We have found that dopamine could be monitored in vivo using online derivatization of dialysate by 6 mM

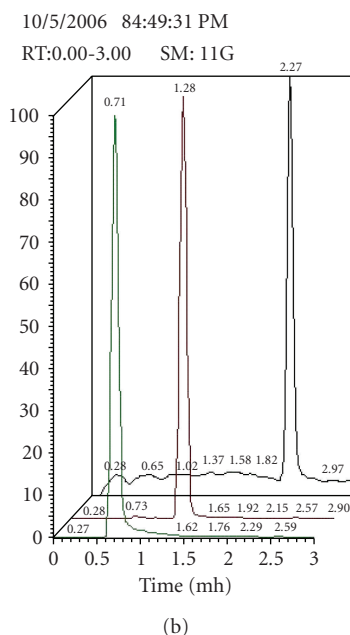
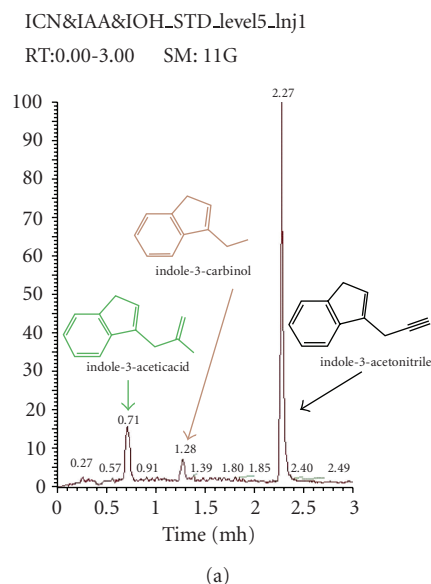


FIGURE 10

naphthalene-2, 3-dicarboxaldehyde, and 10 mM potassium cyanide followed by EKC separation using 6.5 mM sodium dodecyl sulfate (SDS) as the PSP with 2 mM (2-hydroxypropyl)- β -cyclodextrin as a buffer additive. Application of 14.5 kV to a 17.5 cm 10 mm i.d. fused silica capillary allows dopamine to be resolved from other amines in brain samples in 90 seconds. Increased temporal resolution would allow for closer to real time monitoring of rapid changes in dopamine levels; therefore we are exploring methods for increasing the speed of separation. It was found that maximal dopamine resolution obtained at SDS concentrations was just equal to the critical micelle concentration. We have hypothesized that dopamine is strongly partitioned in the phase and faster resolution could be obtained by using lower

concentrations of PSP or a PSP in which dopamine is less retained. To achieve lower micelle concentrations, we are using several generations of the starburst dendrimer poly(amidoamine) as PSPs. Starburst dendrimers are not dependent on noncovalent aggregation and therefore they do not require a minimal concentration to form. We will describe the use of PSP and optimization of other parameters including applied voltage, column length, and buffer composition for improving the speed of dopamine separation for in vivo monitoring.

CONTINUOUS ONLINE MONITORING OF A COPOLYMERIZATION WITH COMONOMERS OF SIMILAR SPECTRAL CHARACTERISTICS

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The analysis of copolymers, for instance, by gel permeation Chromatography (GPC) for parameters which, in the case of block copolymers, might include block length, molecular weight of the blocks, and percent composition, and in the case of random copolymers, might include sequence length and rate of incorporation, is a fairly complex problem. When the two monomers have different chemistries, the use of two detectors may allow some of the above parameters to be calculated by exploiting spectral differences. However, when the two monomers have very similar chemistries and therefore very similar spectral characteristics, as, for example, could be the case in the copolymerization of acrylic monomers, the analysis by conventional GPC is essentially impossible. Here we describe the application of automatic continuous online monitoring of polymerization (ACOMP) reactions to the free radical copolymerization of acrylic comonomers, whose spectral characteristics are very similar. Determination of the instantaneous concentration of each comonomer during the reaction is made possible by incorporating a full spectrum UV spectrophotometer in addition to refractive index, light scattering, and viscosity detectors in a "detector train." Data and results for the copolymerization of butyl acrylate (BA) and methyl methacrylate (MMA) monitored under different starting composition ratios are presented. Continuous conversion kinetics, composition drift, and average composition distribution are calculated from the data, in addition to the evolution of average intrinsic viscosity and weight average molar mass M_w .

QUALITY CONTROL OF SMELL AND TASTE OF FOOD GRADE POLYOLEFIN RESINS WITH AN ELECTRONIC NOSE

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Purpose. Plastic pellets produced for food packaging and water pipes need to be tested for their residual solvents or

monomers responsible for the “plastic smell” on finished products. This test, performed by sensory panel, requires days for sample preparation and a frequent availability of panelists. *Method.* The poster will present how an electronic nose instrument can be used to improve the aroma/flavor testing of HDPE pellets used in water pipe manufacturing. In order to do so, a sample of HDPE coming from each produced batch was analyzed with a quality-control electronic nose. This instrument includes an array of 6 cross-selective sensors to perform the analysis of volatile compounds. *Results.* Results obtained by one leading polyolefin company at its Quality Control lab over a period of several months will show the high level of correlation between instrumental and human assessments (no false positive and 1.3% of false negative, i.e., 1.3% samples were considered as bad by the E-Nose whereas they were acceptable for the human panel). *Conclusion.* This instrumental measurement is now on track for standard approval certification organisms.

AUTOMATION FOR GENOMIC LIBRARY SCREENING ON THE VERSA MINI PCR SETUP WORKSTATION

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Automation and robotics have played a key role in genomics, by relieving researchers of the need to perform repetitive operations on large numbers of samples. Aurora Biomed has introduced the VERSA Mini family of application-specific liquid handling workstations. This modular family offers flexible expansion options for a broad spectrum of applications, enabling scientists to carry out a wide variety of dispensing and contamination-free pipetting tasks in the laboratory. The following study discusses the performance of one of the members of the VERSA Mini family that is designed to perform the task of PCR reaction setup in low- to medium-throughput environments. The study highlights PCR screening of 1500 cloned vector colonies generated from a subtraction cDNA library (pGEMT-EZ) construct. End-point PCR amplification profiles were analyzed using standard gel electrophoresis. The terms of speed and accuracy were compared to the manual performance. The fully automated VERSA Mini workstation, equipped with a single channel, was capable of screening the entire library in a single work-day. The reliable results conclusively showed that this workstation has capabilities in performing automated genomic screening as an alternative to manual performance.

SENSOR ARRAYS FOR WATER-QUALITY MONITORING

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Optical sensor arrays have been fabricated in which microspheres are fixed to the end of optical imaging fibers. A num-

ber of different formats have been developed to use these arrays for multiplexed assays. For environmental monitoring of microbial contamination, nucleic acid testing is being developed. These assays are based on hybridization of either DNA or RNA from the microbes onto appropriately designed probes attached to the microspheres on the array. The arrays have been used to detect contamination of biological agents in sewage, harmful algal blooms in seawater, and infectious agents in drinking water.

OVERVIEW OF CURRENT IN VIVO SAMPLING AND MONITORING TECHNIQUES

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In vivo sampling and monitoring are attractive for several reasons, including reduced labor and materials costs, reduced disturbance of the studied system, reduced potential for error, elimination of separate sampling and monitoring steps, a reduced chance of sample change/loss during storage, and the immediateness of the data, which facilitates timely decision making. The requirements, however, place limitations on the technologies that can be applied for the analysis. This overview is intended to review the current status of in vivo sampling, and identify the strengths and challenges observed by its practitioners.

ROUTINE DRUG SCREENING BY ACCURATE MASS USING LIQUID CHROMATOGRAPHY/TIME-OF-FLIGHT MASS SPECTROMETRY

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LC/ESI-TOFMS offers significant advantages over conventional LC/MS and GC/MS techniques. LC/ESI-TOFMS can be used to measure the monoisotopic mass of pseudomolecular ions to within better than 3 mDa. A second dimension for identification is the degree to which the isotopic pattern (isotopic ratio and accuracy on every isotope) of a detected compound agrees with the theoretical pattern of any analyte (SigmaFit™).

However, as no MRM-type experiments for selectivity are required, a large range of analytes can be screened for at once. In addition, spectral interferences only hinder detection if their m/z values cannot be resolved at resolution of $R > 15000$.

A general drug screening protocol has been developed to detect a wide range of prohibited substances in equine and canine urine using this technique.

Various SPE procedures were used and the extracts were combined and evaluated for their suitability for LC/TOFMS analysis. Both positive and negative ionization modes were employed to give adequate sensitivity for all analytes.

A database was established containing the molecular formula and where known, the retention time of all compounds of interest. Data processing software was developed to create and integrate extracted ion chromatograms of the target pseudomolecular ions in the database within a very narrow mass window (± 3 mDa). Postprocessing software evaluates the spectra by using matching criteria based on mass accuracy of the pseudomolecular ion, that is, SigmaFitTM and retention time. Qualitative findings are then reported automatically in an easily interpretable form.

The screening protocol was shown to meet the required performance specifications for the large majority of the prohibited substances evaluated. LC/ESI-TOFMS analysis can deliver significant savings with respect to sample preparation and analysis time by allowing simplified extraction procedures and analyzing groups of compounds simultaneously that are traditionally handled separately.

AUTOMATED FOURIER TRANSFORM ION CYCLOTRON RESONANCE MASS SPECTROMETRY FOR ULTRAHIGH MASS RESOLVING POWER, PARTS-PER-BILLION MASS ACCURACY, SPEED, AND DYNAMIC RANGE

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This paper is being submitted for the symposium, achievements, and challenges in mass spectrometry. We describe automated analysis of multiple samples by high field (9.4 and 14.5 tesla) Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometry. Mass resolving power is routinely greater than 100 000 at 1 Hz scan rate, and can exceed one million as necessary. Accurate mass can be achieved by use of an internal calibrant (typically ~ 100 parts-per-billion rms error for petroleum samples at 9.4 T) or by combination of external calibration and automatic gain control (AGC) of the number of ions injected into the ICR trap (typically ~ 500 ppb for broadband and ~ 50 ppb for selected ions at 14.5 T). Dynamic range can exceed 1000 : 1 for a single measurement and increases with the square root of the number of averaged measurements. Several MS/MS fragmentation techniques are available and complementary, including collisionally activated (CAD in the linear trap), infrared multiphoton (IRMPD in the ICR cell), and electron capture dissociation (ECD in the ICR cell). Representative applications include petrochemical analysis and proteome profiling with an electrospray robot, and hydrogen-deuterium exchange LC MS for elucidation of protein binding sites (with programmable sample handling). Conceptual design of a 21 tesla ICR magnet is complete and will be described. In addition to field strength, magnet characteristics such as field homogeneity, temporal stability, stray field, size, and cryogenic performance are critical.

ACKNOWLEDGMENTS

The work is supported by NSF DMR-0084173, Thermo Electron Corporation, Florida State University, and the National High Magnetic Field Laboratory in Tallahassee, FLA.

A \$250 AUTOSAMPLER FOR A DART ION SOURCE AND DECONVOLUTION OF COMPOSITE MASS SPECTRA BASED ON EXACT MASSES AND RELATIVE ISOTOPIC ABUNDANCES

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Chemicals are dispersed by numerous accidental, deliberate, or weather-related events. Often, rapid analyses are desired to delineate areas of contamination and to identify dispersed chemicals. Hundreds of wipe samples might be collected from building interiors or outdoor surfaces both before and after any remediation. A DART/oa-TOFMS analyzes compounds in ambient air in seconds on surfaces such as a cotton swab without requiring extraction, extract cleanup, or chromatography. The open design of the ionization region enabled construction of a simple, \$250 autosampler based on N-scale model railroad components and $3' \times 1/4''$ square aluminum rods. Holes spaced $15/32''$ apart from cotton swabs through each of the 14 rods provide for analysis of 1000 samples. Using a rod as the core of a field sampling fixture to provide cotton swab wipe samples that are almost ready for analysis upon receipt might enable one analyst to perform 1000 analyses in one 8-hour shift. In addition, an in-house ion correlation program (ICP) has been used to deconvolute composite mass spectra from multiple analytes on a single cotton swab based on exact masses of monoisotopic ions and the relative isotopic abundances of their +1 and +2 isotopic peaks rather than on the retention time differences of ions provided by chromatography. After mass spectral deconvolution, each of several compounds was identified from its elemental composition. The autosampler, integrated field sampler/autosampler, ICP, and preliminary data will be described.

Notice: The USEPA funded and performed this research. After peer and administrative reviews, this poster was approved for presentation.

APPLICATIONS AND AUTOMATED DATA PROCESSING TOOLS FOR FAST ACQUISITION TOFMS

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In the modern analytical lab there is increasing demand for higher-throughput methods and for analysis of more complex samples. This is, in part, a result of improvements in

instrumentation which has increased our expectations for both the amount of information obtained from a given sample as well as how quickly that information may be garnered. With respect to higher-throughput analyses, recent improvements in HPLC technology have resulted in dramatic reductions in analytical run times with chromatographic peak widths beginning to approach that of GC separations. However, very few detectors are currently capable of providing data acquisition speeds that match these fast chromatographic separations. One of the most useful detectors for such applications has become time-of-flight mass spectrometry (TOFMS), primarily due to its high data acquisition density and its usefulness as an identification tool. TOFMS also boasts high, full mass range sensitivity making it an attractive tool for complex samples in which many unknown compounds must be detected. Unfortunately, when analyzing large numbers of complex samples, a bottleneck is often introduced at the data processing and review stage. In an effort to relieve this bottleneck, promising advances in data processing software have been developed and include both qualitative and quantitative tools. In this presentation, sample applications in the areas of food analysis and metabolomics, demonstrating the benefits of a fast acquisition TOFMS instrument for both high-speed separations and analyses of complex samples, are described. Additionally, integrated software tools for automated data processing, including chromatographic peak finding, mass spectral deconvolution, sample comparison, and automated library searching will be demonstrated.

COMPUTERIZED DESIGN OF SEPARATIONS BY RP-HPLC: THE DRYLAB SOFTWARE

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Computerized design of separations in reversed-phase chromatography (RPC) is today a mature technology which is used worldwide in industrial settings, due to the capability to make potential problems with the RPC method more transparent. The major advantage is however to learn quickly how to avoid experiments of limited quality. As today there is a request to define the quality of an RPC method prior to validation, the importance of the definition, why a certain working point (pH, temperature, %B, etc.) is selected, and how much tolerance should be allowed, is more and more recognized. The paper will compile a few historical milestones on how this technology emerged and will honor Lloyd Snyder, who played a leading role in this development.

ORIGIN OF FRENCH VIRGIN OLIVE OIL REGISTERED DESIGNATION OF ORIGINS PREDICTED BY CHEMOMETRIC ANALYSIS OF SYNCHRONOUS EXCITATION-EMISSION FLUORESCENCE SPECTRA

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The authentication of virgin olive oil samples requires usually the use of sophisticated and very expensive analytical techniques; so there is a need for fast and inexpensive analytical techniques for use in a quality-control methodology. Virgin olive oils present intense fluorescence spectra. Synchronous excitation-emission fluorescence spectroscopy (SEEFs) was assessed for origin determination of virgin olive oil samples from five French registered designations of origins (RDOs) (Nyons, Vallée des Baux, Aix-en-Provence, Haute-Provence, and Nice). The spectra present bands between 600 and 700 nm in emission due to chlorophylls a and b and pheophytins a and b. The bands between 275 and 400 nm in emission were attributed to tocopherols and phenolic compounds, which characterize the virgin olive oils compared to other edible oils. The chemometric treatment (PLS1) of synchronous excitation-emission fluorescence spectra allows one to determine the origin of the oils from five French RDOs (Baux, Aix, Haute-Provence, Nice, and Nyons). Results were quite satisfactory, despite the similarity between two denominations of origin (Baux and Aix) that are composed by some common cultivars (Aglandau and Salonenque). The interpretation of the regression coefficients shows that RDOs are correlated to chlorophylls, pheophytins, tocopherols, and phenols compounds, which are different for each origin. SEEFs is part of a global analytic methodology that associates spectroscopic and chromatographic techniques. This approach can be used for traceability and vindicates the RDOs.

CHEMICAL SENSORS FOR ENVIRONMENTAL MONITORING: ADVANCES IN SURFACE-ENHANCED RAMAN SCATTERING

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In this work, we will show applications of recently developed surface-enhanced Raman scattering (SERS) substrates in the detection of pesticides and water contaminants. Due to the high sensitivity provided by engineered disposable substrates, analyte concentrations as low as ppm-ppb can be detected by SERS with a portable Raman instrument.

Gold-coated regular arrays of micron-size inverted pyramid features are the core of the SERS substrates used in this work. Geometry and size of the textured metal are key to the sensitivity and reproducibility. Typical enhancements and RSD reproducibility of the SERS substrates used in this work are $>10^4$ – 10^6 and $<5\%$, respectively. The substrates have been optimized to give maximum enhancement in the NIR spectral range in order to minimize the fluorescence contributions by molecules or matrices. All the measurements reported in this work have been performed with compact Raman systems at 785 nm. Typical acquisition exposure times used for all analytes are 10–60 seconds.

SERS spectra have been detected for several organophosphate insecticides and herbicides at trace level concentrations. Thorough testing of range concentrations of pesticides in combination with a SERS substrate has shown how SERS could be used in assessing the purity of water.

Performance and practicality of sampling methods will be reviewed to demonstrate the viability of SERS as practical routine analytical technique.

AMINO ACID SCREENING FOR OSTEOPOROSIS IN CHICKEN BY COMPREHENSIVE TWO-DIMENSIONAL GAS CHROMATOGRAPHY

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Osteoporosis is the bone disease that is associated with bone mass loss or the breakdown of the bone matrix which leads to an increased risk in bone fracture. Osteoporosis is not only a bone disease in humans but it also occurs in animals, particularly laying birds like chicken that use a substantial amount of calcium to make the egg shell. A major amount of the calcium used for egg shell formation is intestinal calcium obtained directly from the diet. However, as laying chickens grow older, their ability to absorb calcium from the diet diminishes but their calcium requirement for egg shell formation remains the same. Additional calcium is obtained from the bones by bone resorption through increased hydrogen ion concentration around the bone joints, a process that ultimately results in osteoporosis.

Plasma or serum amino acid screening has clinical significance in different diseases, and studies on bone fracture healing have shown that the serum concentration of free hydroxyproline is correlated with collagen metabolism in bone. Several methods have been developed to measure hydroxyproline, including colorimetric methods, HPLC, and GC/MS. In this project, a GCxGC method has been developed for the screening of amino acids in blood plasma. The GCxGC method was developed primarily to reduce the sample preparation time involved with the other methods from 3–6 hours to under 90 minutes. Another significant benefit of the GCxGC method is the comprehensive screening of the entire amino acid profile including hydroxyproline, which may eventually be useful as a diagnostic indicator for other diseases besides osteoporosis. Details of the experimental protocol and quantitative results comparing the GCxGC and the colorimetric method will be discussed.

OPTIMIZATION OF THE HYBRID MOLECULAR PROBE FOR INTRACELLULAR MEASUREMENTS

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DNA/mRNA detection techniques play an important role in molecular biology. One of the biggest interests in modern bi-

ology is to decipher the transcription and translation processes inside a living cell. One of the tools available is molecular beacons (MBs) which offer several advantages over traditional techniques. However, MBs can be degraded by nucleases inside a living cell, which makes the analysis challenging. To overcome the limitation observed from MBs, we have designed hybrid molecular probe (HMP) for intracellular mRNA analysis in living cells. Briefly, the probe consists of two single strands of DNA which are linked by a PEG (polyethylene glycol) linker. On the 5' end of one strand, a fluorescence donor is attached and to the 3' end of the other strand, another fluorophore acting as an acceptor is attached. When a target that contains the complementary sequences hybridizes to the probe, the two fluorophores are brought within close proximity allowing FRET to occur. PEG keeps the two strands together allowing efficient target hybridization. In order to increase both the selectivity and sensitivity of the probe inside a living cell, we incorporate in the probe design locked nucleic acids (LNAs). Modification with LNAs protects the probe from nuclease digestion, while improving its binding affinity to access highly structure mRNA sequences. The excellent affinity and stability that LNA offers combined with the detection capabilities of the HMP promise an outstanding tool for DNA/mRNA monitoring. Another excellent option to increase the sensitivity of the probe will be the integration of double donors in the HMP. This will increase FRET, therefore higher signal enhancement for the probe upon hybridization with the target. The optimal design of the probe will be applied to prepare probes targeting important cancer-related genes such as MnSOD for the study of the expression of these genes in disease cells at single-cell levels.

PERFORMANCE OF AN NIR MULTIVARIATE OPTICAL COMPUTING-BASED INSTRUMENT ON A SPECTRALLY DENSE BINARY ORGANIC MIXTURE

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Multivariate optical computing (MOC) is a method by which an analyte characteristic of interest is predicted via optical regression using a specialized interference filter called a multivariate optical element (MOE). Previous work in our laboratory has investigated the design and fabrication processes of MOEs and their use in MOC systems in the UV-visible region. More recent studies have probed into the consequences of spectral resolution on the theoretical design of MOEs for application work in the near infrared (NIR) region. The work presented here will examine the actual application of an MOC system designed for a spectrally dense open binary organic mixture of naphthalene and pyrene in the nominal 1675 to 2500 nm region. The actual fabrication process of an appropriate MOE with relation to spectral resolution for this binary organic system will also be included.

MULTIVARIATE CALIBRATION STRATEGIES FOR NONINVASIVE GLUCOSE MEASUREMENTS BASED ON NEAR INFRARED SPECTROSCOPY

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Near infrared spectroscopy in the combination of spectral region is currently being evaluated for use in implementing noninvasive measurements of blood glucose. In our laboratory, transmission measurements through the thin skin on the back of the hand are being studied for their applicability to the noninvasive glucose measurement problem. In initial work, a rat animal model is being employed to simulate measurements in humans. With the rat model, a fiber optic interface is used to make transmission measurements through the skin at the base of the neck of the anesthetized animal. Comparisons of spectra collected in this manner to analogous spectra collected from the back of the hands of human subjects reveal a close spectral match. By acquiring spectra continuously as glucose excursions are induced in the rat, datasets are being obtained for use in exploring methods to quantify glucose in these noninvasive spectra. Challenges to a successful calibration include the presence of many sources of measurement variance, as well as the difficulty of extracting the small glucose signature from the many overlapping spectral components of the tissue matrix. In this presentation, multivariate calibration methods will be explored for their utility in building robust predictive models for glucose in noninvasive spectra. What of particular interest in this work is the performance of hybrid calibration methods that combine the spectra of known tissue constituents with abstract components derived empirically from spectral background measurements. To evaluate these calibration methods, datasets acquired from multiple rats will be used. Issues to be addressed include the ability to apply calibration models across different rats and the ability to overcome the various sources of data variance associated with the noninvasive measurements.

CHARACTERIZATION AND MODELING OF CONDUCTING COMPOSITE ELECTRODES

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Conducting composite electrodes are versatile devices for biomedical applications showing good biocompatibility, low cost, and ease of construction. They can be bulk modified with stabilized enzymes to make biosensors and electrodes for biofuel cells [1]. Low sensitivity to flow conditions arises from the microelectrode array-like behavior but this is accompanied by high capacitance and bulk resistance which can blur the voltammetric detail [2].

We have undertaken a systematic investigation of the effects of composition and formulation on the voltammetric

behavior and nonfaradaic properties. Monodisperse glassy carbon balls have been used to simplify the modelling and the relative importance of the patterns of surface conductivity and bulk 3D connectivity has been investigated. Three-dimensional numerical models based on percolation theory have been constructed which allow calculation of the distributed resistances in the composite and enable qualitative prediction of the voltammetric properties.

Voltammetric results showed a bias of E^0 (half-reaction potential) and an unstable i_l (diffusion limiting current) for conducting composite electrodes. AC impedance showed dramatic variation of R_{ct} (charge transfer resistance) and C_d (double layer capacitance) with composition and thickness. Comparison with carbon fiber arrays enabled separation of the effects of a distributed interface from three-dimensional disparities in the conductance. Key findings are as follows. The distributed resistance in electrodes results in shifts in E^0 and the overlap of diffusion layers leads to a potential dependent i_l .

Numerical results show the shifts in E^0 and i_l which are in agreement with experiments.

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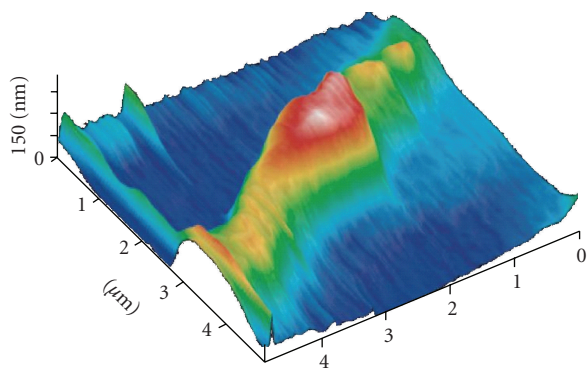
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SIMULTANEOUS ELECTROCHEMICAL, NEAR-FIELD OPTICAL, AND TOPOGRAPHIC IMAGING BY USING SCANNING ELECTROCHEMICAL/NEAR-FIELD OPTICAL/ATOMIC FORCE MICROSCOPY

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Simultaneous scanning electrochemical/near-field optical/atomic force microscopy (SECM/NSOM/AFM) was developed and applied to the local mapping of a living cell. A novel bent-type probe was applied, which consists of an optical fiber surrounded by a gold ring electrode, and an outermost electrophoretic insulating polymer. The tip of the bent-type probe was fabricated by a focused ion beam (FIB) process having an aperture size of about 100 nm. Therefore, the tip has both near-field optical and electrochemical properties. The electrochemical properties were evaluated by cyclic voltammetry in 10 mM potassium ferricyanide solution, containing 500 mM of KCl. The cyclic voltammogram showed a sigmoidal response with a diffusion-limited current I_d of 0.73 nA. This indicates an electrode diameter in the nanometer order. To control the distance between the probe and the substrate, an optical feedback mechanism was employed. In the SECM/NOSM/AFM setup, the



Topographic imaging of PC12 neuritis

FIGURE 11

electrochemical response at the tip electrode was monitored with a potentiostat and the near-field optical signal with an avalanche photo detector through a lens, and the atomic force was detected via an optical feedback system. The imaging capability of the SECM/NSOM/AFM technique was confirmed by simultaneous acquisition of electrochemical, near-field optical, and topographical images of an interdigitated array electrode ($1\ \mu\text{m}$ gold bands spaced by $2\ \mu\text{m}$ glass) in an aqueous redox-active solution containing $3.0\ \text{mM}$ hydroxymethylferrocene and $500\ \text{mM}$ KCl. Simultaneous imaging with SECM/NSOM/AFM revealed that the images obtained in the SECM mode (positive feedback while scanning over gold layers and negative feedback over glass) and the NSOM mode corresponded to the ones observed in the AFM mode. The resolution level of each imaging mode was found to be $100\ \text{nm}$ for SECM, $80\ \text{nm}$ for NSOM, and $100\ \text{nm}$ for AFM. Therefore, all three modes are useful for the local mapping of the biochemical activity of a living cell. We selected PC12 cells, which are often used as a model for neurons. We demonstrated that SECM/NSOM/AFM can be used for imaging neuritis ($1\text{--}5\ \mu\text{m}$) of differentiated PC12 cells and for detecting varicosities (about $100\ \text{nm}$ wide) related to the release of catecholamines in the neuritis (Figure 11).

BIOMONITORING FOR NITRO-POLYCYCLIC AROMATIC HYDROCARBON METABOLITES AND DIESEL EXHAUST BIOMARKERS IN HUMAN URINE BY GAS CHROMATOGRAPHY/HIGH-RESOLUTION MASS SPECTROMETRY

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Polycyclic aromatic hydrocarbons (PAHs) and nitro substituted PAHs (NO_2 -PAHs) are a group of environmental pollutants formed during incomplete combustion. NO_2 -PAHs are especially important since they have been reported to account for over 50% of the direct mutagenicity in ambient air. Some of them are estimated to be up to four orders of magni-

tudes more mutagenic and ten times more carcinogenic than PAHs. The main source for NO_2 -PAHs in the atmosphere is diesel exhaust. After entering the body, NO_2 -PAHs are metabolized to aromatic amines before conjugation and/or excretion in urine. Certain amino-PAHs have been suggested as possible biomarkers for diesel exhaust exposure, such as 3-aminobenzanthrone (3-abz) and 1-amino-pyrene (1-apyr).

The Centers for Disease Control and Prevention (CDC) is currently developing methodology to measure amino-PAHs and related compounds in human urine. The method involves acid deconjugation, liquid-liquid extraction, and analysis by gas chromatography/high-resolution mass spectrometry (GC/HRMS). Deconjugation using different acids has been evaluated and compared to enzymatic deconjugation. After acid hydrolysis, the sample mixture was washed with pentane, basified with sodium hydroxide, and extracted with dichloromethane/pentane (vol 1:1). The final extracts ($\sim 8\ \text{mL}$) were evaporated under a gentle stream of nitrogen, reconstituted in toluene, and analyzed by GC/HRMS in the selective ion monitoring mode. Complete separation was achieved using an RTX-35 amine column and quantification was accomplished using four ^{13}C -labeled amino-PAHs as internal standards, spiked into the urine samples before extraction. This method will be applied in the future for the analysis of samples from the National Health and Nutrition Examination Survey (NHANES), performed by CDC to assess exposure of the US population to environmental pollutants.

LC/EI/MS FOR THE IDENTIFICATION OF MEDICINALLY ACTIVE NATURAL PRODUCTS IN ACORUS CALAMUS

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An investigation into the medicinally active chemical constituents of prairie plants utilized by the Plains Indians is ongoing in our laboratories. Our current investigation is on the rhizomes of *Acorus calamus* (sweet flag). The rhizomes contain aromatic oil that has been used medicinally since ancient times and *A. calamus* is cultivated in Asia for this reason. The rhizomes are considered to possess antispasmodic, carminative, and anthelmintic properties and also used for the treatment of epilepsy, mental ailments, chronic diarrhea, dysentery, bronchial catarrh, intermittent fevers, and glandular and abdominal tumors. They are also employed for kidney and liver troubles, rheumatism, sinusitis, diabetes, and eczema. The methanolic extracts of *A. calamus* rhizomes were investigated by reversed-phase liquid chromatography electron impact mass spectrometry (LC/EI/MS) and gas chromatography mass spectrometry (GC/EI/MS). The polar constituents are of interest since Plains Indians frequently made teas or other preparations utilizing aqueous extractions. While LC/ESI/MS works well for polar molecules of known identity, the difference in fragmentation patterns among ESI instruments and the lack of extensive ESI libraries make the identification of the components in a complex mixture very difficult. This method permits the rapid assessment

of plant extracts for the presence of medicinally active compounds with a minimum of prepurification. In LC/EI/MS, the effluent from an HPLC is introduced into an electron impact ionization source giving typical and well-understood EI spectra. The advantage to acquiring EI fragmentation data from an LC separation lies within the subsequent ability to use existing deconvolution and search programs to match results with well-established and commercially available EI mass spectral databases.

ONLINE ELECTROCHEMICAL SYNTHESIS AND ANALYSIS OF SMALL MOLECULE METABOLITES IN DRUG DISCOVERY

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An important step in small molecule drug discovery and development is the synthesis of substances derived from investigational compounds. These related substances include products and metabolites that are commonly a result of oxidative processes. Several studies utilizing electrochemical (EC) flow cells online with high-performance liquid chromatography (HPLC) and mass spectrometry (MS) have demonstrated that electrochemically derived products often correspond to biological metabolites and chemical degradants. It is of interest to the drug-discovery process to be able to synthesize these oxidative metabolites in microgram quantities for further evaluation.

The objective of these studies is to examine the use of controlled-potential electrolysis in flowing solution as a means of small-scale synthesis to facilitate pharmaceutical analyses in discovery and development. The experimental apparatus consisted of an HPLC system, where inline EC flow cells comprised of large surface area flow through working electrodes, followed by UV and MS detectors. Results using microgram quantities of starting materials have indicated selective and semiquantitative formation of oxidative products such as N-dealkylation of amitriptyline. Reaction cells were placed before the HPLC columns to allow separation, concentration, and purification of reactants and products. Results from optimization of conditions for product yield and purity will be discussed in the context of electrode size, concentration of starting material, cell potentials, and flow rates.

AUTOMATED CAPILLARY COLUMN IMMUNOASSAY FOR RAPID DETECTION OF MITOCHONDRIAL

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Proteomics is increasingly used to gain greater understanding of the many functions of mitochondria in metabolism and metabolic disease. Increased use of proteomics has been facilitated by simplifications in protein identification by methods such as 2D-PAGE, MALDI-MS, and Edman sequencing.

Immunochemical detection has high specificity and sensitivity, and large sample throughput. However, these detection methods also require multistep liquid handling and are time-intensive. The use of antibody/antigen-modified capillary columns presents advantages over traditional immunochemical methods. The capillary geometry improves assay kinetics due to the higher surface-to-volume ratio and restricted diffusion of compounds. In addition, capillary columns are more suitable for automation, and consumption of immunoreagents is minimized due to the small dimensions of capillaries. With the realization of significance of proteins in metabolism and metabolic diseases, a high-throughput instrument for the detection of proteins is of increasing importance. A five-channel prototype instrument was developed for multisample, highly automated, rapid detection of proteins in serum samples using optical detection. For detection of proteins, capillary columns were modified with a primary antibody. After capture of proteins, a secondary antibody was applied and detected using enzyme labeled anti-IgG and the substrate. The assay can be completed in less than 3 hours per 10 samples. For vinculin, a linear detection range of 0~18.8 $\mu\text{g/mL}$ (0~160 nM) was obtained with a lower limit of detection (LOD) of 0.5 $\mu\text{g/mL}$ (4.3 nM). For cytochrome c, a linear detection range of 0~0.5 $\mu\text{g/mL}$ (0~41.7 nM) was obtained with an LOD of 0.01 $\mu\text{g/mL}$ (0.83 nM). All the antibodies used were evaluated with ELISA prior to the capillary column tests. It was demonstrated that the immunosensor has sufficient sensitivity to detect the target proteins in lymphocyte samples.

APPLICATION OF THE ELECTRONIC TONGUE TO ANTIBIOTICS DETECTION IN MILK

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Development of rapid methods for routine food analysis and quality control is an urgent task. For example, determination of the antibiotics' concentration in milk is an essential part of milk analysis performed in the dairy plant. Reasons for this are twofold. Firstly, the content of antibiotics in milk should not exceed limits that are allowed for human consumption. Secondly, if the milk used for production of fermented dairy products contains too much antibiotics, they may inhibit growth and development of the added bacterial culture and, consequently, the quality of the final product will deteriorate. Currently used procedure requires milk inoculation with bacterial culture, incubation during three hours, and consequent determination of the amount of the produced lactic acid by the titration. Since activity of the bacterial culture depends on the amount of antibiotics present in milk, concentration of lactic acid produced during milk fermentation is correlated to the antibiotics content. Evidently, there is an interest in the developing of much faster and less laborious procedure for milk quality control.

Electronic tongue multisensor systems appear to be promising tool for such task.

The present work is devoted to the development of an analytical procedure for ET for rapid antibiotics' detection in raw milk.

Electronic tongue developed in the Laboratory of Chemical Sensors of Saint Petersburg state University was used for milk measurements.

Sample preparation consisted in addition of different quantities of penicilline to milk, milk inoculation with bacterial culture, and incubation.

PCA score plot of measurements made in milk samples that contained 2 ppb of penicillin after 0, 20, 40, and 60 minutes of incubation is shown in Figure 12. The difference between inoculated milk samples containing 0 and 2 ppb of penicillin became pronounced already after 20 minutes of fermentation.

During the next experiment, milk samples containing 0, 2, 3, and 10 ppb of penicillin and inoculated by bacterial culture were measured using the ET. Calibration models with respect to the penicillin content in milk were made using PLS regression for each incubation time. An acceptable calibration model was obtained using the data from the measurements made already 30 minutes after inoculation of milk. RMSEC was found to be 0.02 and RMSEP was found to be 0.13 in this case.

The ET was demonstrated to be viable and fast method of antibiotics detection in milk.

DESIGN OF A BIOSENSING SYSTEM BASED ON AN INTRINSICALLY UNSTRUCTURED PROTEIN AS A BIOLOGICAL RECOGNITION ELEMENT

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Intrinsically unstructured proteins classify a group of proteins lacking global tertiary structure. It is found that upon binding to a target molecule, many of these unstructured proteins inductively fold. This conformational change coupled to the binding event can be utilized in the development of detection assays for target molecules by conjugating a transduction moiety to the unstructured protein. A small fragment of the BRCA1 tumor suppressor protein (residues 219–498) has been found to bind another tumor suppressor protein. This region of BRCA1 has been classified as unstructured via circular dichroism (CD) spectroscopy, as seen in Figure 13. A strong dip at approximately 205 nm confirms the unstructured nature of the peptide. A conformational change in the BRCA1 region results from the p53 (residues 355–393)/BRCA1 binding event. This conformational change was characterized by the CD spectra which shows that the disordered content of the BRCA1 diminishes upon binding of the p53 peptide to the BRCA1 peptide. Observed quenching of the BRCA1 intrinsic tryptophan fluorescence upon addition of p53 is also evidence of this conformational change. Our hypothesis is that a similar quenching

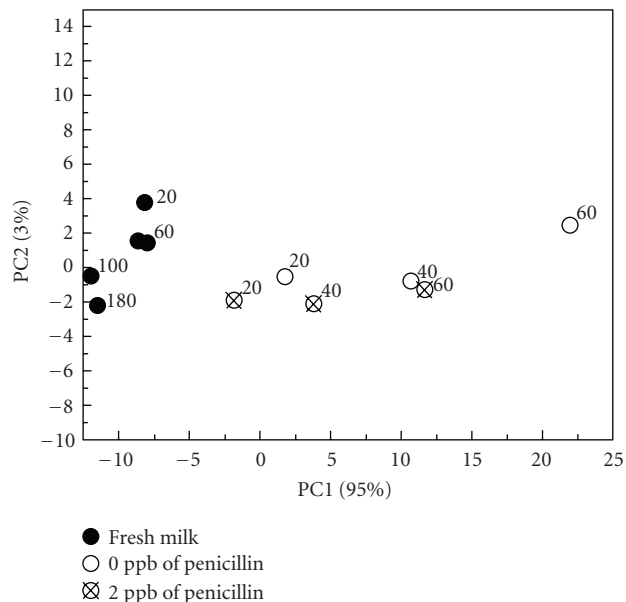


FIGURE 12

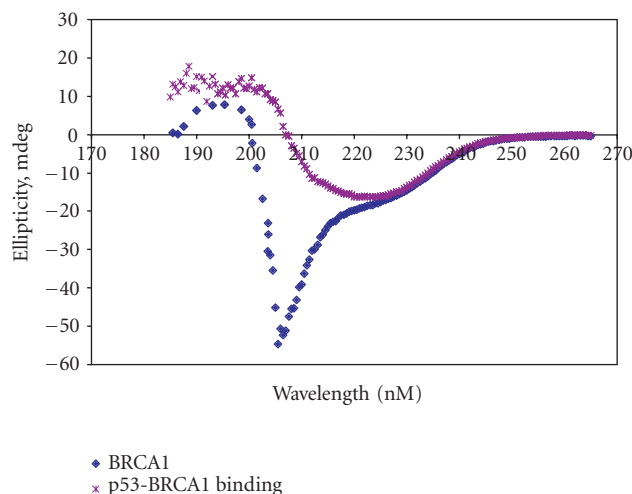


FIGURE 13

can result in the fluorescence of a probe attached to BRCA1 upon addition of p53. This quenching in fluorescence can then be related to the amount of p53 present in the sample. To develop a fluorescence-based detection assay, tetramethyl rhodamine-5-maleimide (TMR) was conjugated through the thiol moiety of cysteine residues on BRCA1. The BRCA1-TMR conjugates was characterized in terms of its fluorescence properties. Upon mixing of p53 and BRCA1-TMR conjugate, a change in the fluorescence signal was observed, which was correlated with the amount of p53 present in the sample. Control studies were performed using molecules that do not interact with BRCA1. This biosensor based on unstructured proteins may be applied to numerous unstructured protein-target binding pairs.

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IDENTIFICATION OF VOLATILE COMPOUNDS AS INDICATORS OF SALMONELLA CONTAMINATION OF BEEF USING SPME-GC/MS

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Salmonella typhimurium contamination in meat (beef) is a critical concern. There is a need to develop rapid and reliable methods for detection of *Salmonella* status in beef to ensure consumer safety. Thus, a multidisciplinary project is underway to develop intelligent sensors for rapid detection of *Salmonella* contamination in beef based on olfactory sensing of headspace gases. Since in real-world conditions, other spoilage bacteria could also grow along with *Salmonella*, it was considered a priority to identify the volatile compounds due to *Salmonella* contamination alone.

Beef strip loins were aseptically transferred to sterile headspace vials, inoculated with *Salmonella typhimurium* and stored at 20°C for 4 days. The volatiles generated in the headspace of the vials were investigated each day using headspace solid phase microextraction (HS-SPME) in combination with gas chromatography-mass spectrometry (GC-MS). Microbiological analyses of both inoculated and control samples were also carried out to obtain *Salmonella* population.

HS-SPME/GC-MS detected several volatile compounds in the headspace of beef samples, inoculated with *Salmonella typhimurium*, namely, carbon dioxide, ethanol, acetone, carbon disulphide, 2,3-butanedione, ethyl acetate, acetic acid, 3-hydroxy-2-butanone, dimethyl disulphide, and also a few hydrocarbons. However, these compounds were also detected in the headspace of the control samples.

Acetic acid, ethanol, carbon dioxide, and 3-hydroxy-2-butanone were the most important compounds identified in the study. Two-way ANOVA with storage time and sample source (inoculated and controled) as the model factors were used to analyze the data. The F-tests (Fisher's variance ratio) for the main effect of the sample source established acetic acid, ethanol, and carbon dioxide as potential useful indicator compounds for *Salmonella*-contaminated fresh beef samples. Good linear correlations were found between the logarithm of average peak area response and *Salmonella* count (\log_{10} cfu/g) for all the four compounds, that is, acetic acid ($r = 0.99$), carbon dioxide ($r = 0.99$), 3-hydroxy-2-butanone ($r = 0.99$), and ethanol ($r = 0.89$).

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SHOULD YOUR AUTOSAMPLER JUST SIT AND WAIT? INTELLIGENT INSTRUMENT CONTROL ALLOWS SHARING HPLC MODULES ACROSS SYSTEMS

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In traditional HPLC configurations, some of the modules are not very well utilized. For example, the autosampler is idle for most of the time and a thermostatted column compartment could hold more than one column. The situation can be improved if these modules could be utilized in more than one system.

By using a dual gradient pump (two independent pumps in one enclosure) and an additional detector, a system suitable for "parallel chromatography" can be configured. In this setup, the column compartment and autosampler are shared between two HPLC systems (the two pumps are assigned to their own system). This approach nearly doubles the throughput and can be used for isocratic as well as gradient methods. Another advantage is that this gain in productivity can be reached without modifying and revalidating the existing analytical method. However, control of such a complex system requires powerful software.

In this poster, we present an intelligent software solution for controlling shared devices. From the user's point of view, the instruments behave as two independent HPLC systems, although physically only "one and a quarter" systems is present. The software automatically takes care of the sharing process (e.g., manages exclusive access to the autosampler), and handles potential issues (e.g., in case of problems with one of the systems, the other one can continue).

With a relatively little additional cost, intelligent module sharing between HPLC systems can almost double the productivity of a traditional HPLC system, without the need for modifying and revalidating existing analytical methods.

COMPUTER-AIDED DIAGNOSTICS AND TROUBLESHOOTING OF HPLC INSTRUMENTS

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HPLC is often perceived as a complex technique. Even the most reliable hardware will eventually fail. The amount of time needed for troubleshooting and repair strongly influences the uptime of the instrumentation and, as a result, overall laboratory productivity. Therefore it is important to quickly find the source of the issue and resolve it.

Diagnosing instrument problems is not easy. Although diagnostic tests are often built on simple principles (e.g., watching a pressure drop to find leaks), the actual procedure is in most cases rather complex. The user must observe many side conditions, and the results are sometimes difficult to interpret.

In this poster, we present an advanced approach of computer-aided diagnostic tests and online troubleshooting guides. The module-based tests are intuitive and easy to use. The user is guided step by step through the procedure. The results are displayed both graphically and numerically with pass/fail evaluation. In addition, the results can be printed and/or stored. Once the issue is identified, an online troubleshooting guide helps to provide immediate remedy.

The advanced module-based diagnostics and the online troubleshooting guides ensure minimum instrument downtime for increased productivity.

RAPID AND AUTOMATED EXTRACTIONS OF DRUGS AND METABOLITES USING DISPOSABLE PIPETTE EXTRACTION

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Disposable pipette extraction (DPX) is a rapid method that utilizes solid phase extraction in a pipette tip. The sorbent is mixed with the sample solution using DPX, and this leads to reproducible and rapid extractions that take less than approximately 2 minutes to perform. Another main advantage of DPX is that the method can be readily automated with most commercial liquid handlers.

DPX methods for the extraction of drugs and metabolites from serum, urine, and saliva samples are introduced. Also, automated solutions for hands-free sample preparation are presented. Examples of rapid DPX extraction combined with rapid chromatography are also presented, and the ability to use these methods for faster, less expensive, and more accurate results than immunoassay methods is discussed. Also, examples of high-throughput screening are provided.

MONITORING SE EXCRETION IN URINE AFTER INGESTION IN BRAZIL NUT BY ELECTROTHERMAL ATOMIC ABSORPTION SPECTROMETRY

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Selenium is recognized as essential element and has a large number of biological functions in human organism. The most important action is its antioxidant effect because Se is in the active center of glutathione peroxidase enzyme. The Food and Nutrition Board of United States Research Council proposed an appropriate and estimated safe daily intake of Se for healthy man and woman adults of 70 and 55 $\mu\text{g}/\text{day}$, re-

spectively. The toxicity causes chronic poisoning called alkali disease in livestock. On the other hand, Se deficiency promotes Keshan and Kaschin-Beck diseases, mainly, in the Chinese population.

The major source of Se for the general population is food, such as Brazil nuts. However, the Se content of food is highly dependent on the amount of Se in the soil. In the case of Brazil nut, Se concentration of Acre to Rondonia and Manaus to Belem ranges to 0.03–32 $\mu\text{g}/\text{g}$ and 2–512 $\mu\text{g}/\text{g}$, respectively. Studies showed that half of a nut with Se concentration of 126 $\mu\text{g}/\text{g}$ contains the per-day quantity recommended for a person of 70 kg. Therefore, the aim of this work was to evaluate the excretion of Se in urine after ingestion of a portion of Brazil nuts. Six volunteers (3 men and 3 women) consumed 5 nuts (approximately 18 g) and their urines were collected during 24 hours. The first urine samples were collected immediately before the ingestion of the Brazil nuts (8:00 am) and the others were collected every 1 hour, until 9:00 pm. The first urines of the day after were also collected for all volunteers. In all urine samples, Se was determined by electrothermal atomic absorption spectrometry (ETAAS). Additionally, creatinine was also determined according to reaction of creatinine with picrate in alkaline solution to form a red-orange product that can be properly detected by UV-vis spectrophotometry ($\lambda = 510 \text{ nm}$). The creatinine concentrations in the urine samples ranged from 1.3 to 2.1 g/L.

The maximum concentrations of Se were observed between 4 and 6 hours after eating the Brazil nuts. The baselines were getting after 10 hours. For 4 volunteers, the Se concentration ranged from 61 to 130 $\mu\text{g}/\text{L}$. For the other two volunteers, it ranged from 658 to 714 $\mu\text{g}/\text{L}$.

It is important to point out that sex, age, and diet differences can influence the Se excretion.

CHEMOMETRIC-BASED QUANTIFICATION OF A FAST LIQUID CHROMATOGRAPHY-DIODE ARRAY DETECTION METHOD FOR A MULTICLASS SAMPLE OF COMMON DRUGS OF ABUSE

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Drug determination requires optimized chromatographic methods in order to quantitatively determine the identity and amount of drugs in a biological sample. However, many methods are either specific for a class of compounds or take upwards of twenty to thirty minutes to resolve a multiclass sample. A need for a fast, simple, multiclass analysis is clearly present in this field. Using chromatographic figures of merit such as multivariate selectivity and applying parallel factor analysis (PARAFAC) to three-way data, we seek to develop and optimize a fast liquid chromatography-diode array detection (LC-DAD) method that is able to distinguish and quantify a synthetic 20-component urine sample containing common drugs of abuse and their metabolites. This method

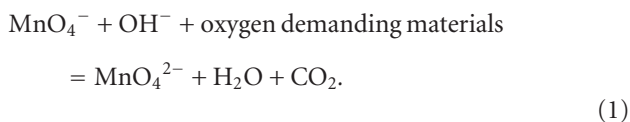
can then be transferred to the more sensitive LC-mass spectrometry (MS) for conclusive results.

FAST ANALYSIS OF COD VALUE OF SEAWATER BY SPECTROPHOTOMETRY

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The chemical oxygen demanding (COD) value is the common index of total organic pollutants in seawater. This value was tested by the oxidizing reaction of organic pollutants in seawater under alkali and heating conditions with a known amount of superfluous potassium permanganate. K_2MnO_4 was generated quantitatively. The concentration of MnO_4^- , which indicates the COD value, is measured by a 721 spectrophotometer at the wave length of 430 nm:



The mixture of the potassium biphthalate and EDTA was employed as standard material to give out the criterion curve of COD. The experimental results show that when the COD in seawater is between 0.5 and 5.0 mg/L, the method is fast, sensitive, reliable, and simple.

OPTIMIZATION OF PURGE AND TRAP SYSTEM CONDITIONS FOR THE DETERMINATION OF VOC IN DRINKING WATER

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There are several demands and requirements imposed on chemists performing volatile organic analysis in today's environmental laboratory. The first and most important thing is that the analysis must be performed in compliance with USEPA methodologies. Next, there is a continued trend to achieve lower levels of detection. Purge and trap technology offers a method for the extraction and concentration of these VOCs from multiple matrices such as waters and soils. US EPA method 524.2 measures a wide range of compounds in drinking water with typical detection levels at sub-ppb-levels coupled with stringent quality-control requirements. Although drinking water is a clean matrix, there are still several analytical concerns which must be considered.

This paper will present the optimum purge and trap system parameters particularly the water management conditions used in compliance with the method 524.2 to provide the necessary sensitivity, linearity, and accuracy. Analytical results including calibration factors, method detection lim-

its, and reproducibility data will be shown meeting all quality assurance criteria.

A RAPID AND AUTOMATED ANALYSIS OF ASPARTAME IN DIET BEVERAGES

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A rapid assay for the determination of aspartame in diet beverages was automated on a discrete wet chemistry analyzer. The method is customizable to different calibration ranges to accommodate different matrices. This method replaces a slower HPLC method currently in use. A large increase in efficiency was seen from the HPLC method, which requires a 10-minute pretreatment of the sample, to the discrete method where the pretreatment was automated to 30 seconds per sample. Two calibration ranges will be presented with supporting data at both high and low levels for diet beverages.

The new discrete method automates an existing manual enzymatic method using the analyzer to perform all dispensing, dilutions, mixing, incubating, and photometric readings of the reactions. The detection of aspartame requires three enzyme reactions, after the initial treatment at high pH, to remove the methyl group from the L-phenylalanine residue.

The method uses a total volume of 111 μ L per test, blanking of each sample to correct for color or turbidity, and two incubations totaling 7 minutes. The reaction cuvette is automatically discarded after the photometric reading is complete. The use of disposable cuvettes prevents carryover contamination from becoming a factor in the analysis. Different calibration ranges specific to matrix are recommended for best accuracy.

AN IMPROVED METHOD FOR HIGH-THROUGHPUT ANALYSIS OF ISOFLAVONES IN SOY-CONTAINING FOODS AND DIETARY SUPPLEMENTS BY RP-HPLC

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Soybeans are known to contain the nine isoflavone glycosides, daidzin, glycitin, genistin, 6''-O-Malonyldaidzin, 6''-O-Malonylglycitin, 6''-O-Malonylgenistin, 6''-O-Acetyldaidzin, 6''-O-Acetylglycitin and 6''-O-Acetylgenistin, and their corresponding aglycones, daidzein, glycitein, and genistein. Soy isoflavones are structurally similar to endogenous estrogens, and demonstrate both estrogenic activities and antiestrogenic activities. Soy-containing foods and dietary supplements are widely consumed for putative health benefits (e.g., reduction of osteoporosis, relief of menopausal symptoms, breast cancer chemoprevention). However, there is also some concern that excessive intake of soy isoflavones

could stimulate tumor growth in women with estrogen-related cancers. Recently, increasing interest has focused on the determination of the levels of isoflavones in soy-derived foods and estimation of isoflavones in the daily intake.

This study developed a high-throughput method for quantifying isoflavones in soybeans, soy foods, and dietary supplements by RP-HPLC. Twelve isoflavones were completely resolved within 10 minutes by using a silica-based C18 column specially designed for separation of highly polar compounds (Hydrosphere C18 column, 50 × 4.6 mm i.d., 3 micron, YMC Co., Ltd.), with a gradient elution of acetonitrile and water containing 3% acetic acid. The effective sample preparation conditions were also determined, comparing the effects of temperature, solvent, and extraction time.

AUTOMATED APPLICATION SWITCHING IN FOOD AND BEVERAGE ANALYSIS FOR INCREASED USE TIME OF HPLC INSTRUMENTATION

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Laboratories regularly have to analyze a variety of parameters from the same sample set. Often more than one HPLC method is needed to obtain the required results. Various strategies may be adopted to eventually increase productivity.

Traditionally, the user runs one method and manually switches to the other method. The manual method changeover makes it impossible to consecutively run the methods unattended over night or the weekend. This results in increased labor time compromising productivity.

Another approach would run each method on a dedicated HPLC system at the same time. This approach requires two separate HPLC systems and enough bench space. Both systems have to be operated simultaneously by possibly two different users.

An alternative concept-automated application switching is based on a novel dual gradient system occupying the same bench space as one system. The user sets up both methods. The system runs the first method, then automatically switches to the other method without any additional user interference. This approach frees operator time, increases system usage time, allows automation, and thus boosts productivity.

This poster demonstrates implementation and benefits of using automated application switching in the food and beverage market. Two typical food and beverage routine analyses of the same sample are used for this purpose.

SUCCESSFULLY AUTOMATING THE SMALL CHEMICAL LABORATORY

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Smaller laboratories often do not have the luxury of enough routine samples for managers to feel that there is adequate justification for the capital investment of an autoanalyzer. Continuous flow analyzers, though highly flexible forms of automation, can be expensive because of the time required in method changeovers. Discrete analyzers open the door to automation for smaller labs. Discrete analyzers are very cost-effective because a single instrument can analyze almost every wet chemistry parameter.

With the versatility and simplicity of discrete analyzers, it is possible to economically analyze samples of widely varying matrices without the need for batching. Samples can be analyzed when received, or immediately after preliminary digestions and extractions. This poster illustrates the ease of automated measurement of multiple analytes from different sample matrices on a Discrete Analyzer introduced by OI Analytical.

TRANSPORTABLE AUTOMATED DIESEL FUEL ANALYZER

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Recently, high price of crude oil is big issue all over the world. The situation makes nonoil producing countries harder. Most governments in these countries impose high taxes on oil products such as gasoline. For this reason, many illegal fuels are traded and most of those illegal fuels have low quality. These illegal fuels may bring troubles to vehicles and cause environmental problems including air, water, and/or soil pollution. We have developed a transportable automated diesel fuel analyzer for field detection of impurities in diesel fuel. This diesel analyzer, located inside a car, consists of automatic pumping system and PDA spectrophotometer to check the quality of diesel fuel using colorimetric quantitative method at the gas station. If impurities are mixed in, reagent will cause the color change of the diesel in about 1 minute during refueling process. A laptop computer with analysis software would identify and determine the concentration of impurities (Figure 14). Three peristaltic pumps are used for sample transportation and two syringe pumps for precise sample volume metering. The injected diesel and reagent are transferred to a mixing vessel with stirring assembly by those pumps. A photodiode array technology provides unmatched speed of about 1 second for measuring entire visible area and stability with nonmoving parts in the spectrograph. The integrated two-position automatic referencing stage ensures reproducible and accurate results by automatic baseline correction.

From injection to draining of diesel, all processes are automatically controlled by a laptop computer via USB communication. This system will help fuel producing companies and/or government agencies involved in controlling environment or checking fuel quality. Results are shown in Table 1.

TABLE 1

	Absorbance	Conc. (ppm)	Function	R ²	Measurement time
1	-0.0001	0	Conc. = A × 64.4374 + 0.3765	0.9963	2006-07-27, 8:58 am
2	0.0351	2.5	—	—	—
3	0.0975	7.5	—	—	—
4	0.2321	15	—	—	—

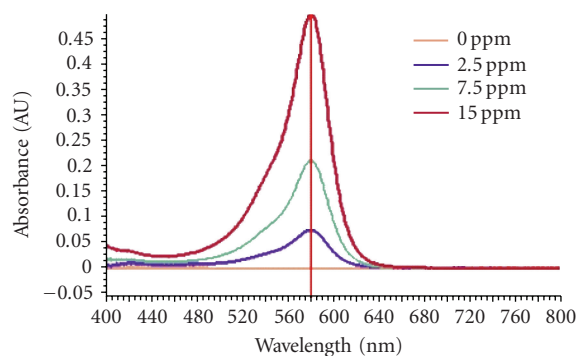


FIGURE 14

ELECTRONIC NOSE AND NEW TECHNOLOGIES OF E-NOSE TO IMPROVE ODOR/FLAVOR ANALYSIS FOR FORMULATION DEVELOPMENT AND ROUTINE QUALITY CONTROL

Jean-Christophe Mifsud

Sensor arrays systems such as electronic noses and electronic tongues have generated a great level of interest in the analytical laboratories of the world's leading food, flavors and fragrance, packaging, and pharmaceutical companies, as well as in the environment as a fast, simple, and reliable method of aroma/VOC analysis. Over the last few years, the sensor arrays systems have demonstrated their ability to provide industrial solutions and the ability to transfer expert knowledge from the R&D and trained sensory panels into a production environment for quality assurance and control. Flavors quality, intensities, and taste quality control attracts an increasing level of attention from many companies. Those systems reduce the needs for sensory evaluation or GC/HPLC and provide added safety when performing taste or olfaction assessments. They are simple to use and extremely fast.

MICROFLUIDIC DEVICES FOR CHEMICAL MONITORING OF LIVING CELLS IN CHIPS: PARALLEL AND LONG-TERM MEASUREMENTS

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Monitoring the environment around living cells is a key type of experiment in physiology, pharmacology, cell biology, and drug discovery. Microfluidics offers the potential to revolutionize this type of experiment by integrating cell culture and

chemical analysis systems with automation, high throughput, and precise control. In our laboratory, we have explored this concept by developing microfluidic chip that can monitor hormone secretion from living cells. We are using islets of Langerhans and monitoring insulin and glucagon secretion as a model system. In the chip, single islets are perfused with cell culture media and the perfusate is analyzed by an on-chip, electrophoretic immunoassay for insulin or glucagon. We have demonstrated that these chips can be used continuously without operator intervention for 24 hours (corresponding to 17 000 assays). This long-term monitoring will be important for monitoring slow changes in secretion. We have also developed a chip that allows up to 15 islets to be monitored independently for high-throughput operation. These chips have application for fundamental diabetes research and as clinical tools in evaluating transplantable islets.

ONLINE ELECTROCHEMISTRY TO STUDY THE DYNAMICS OF FLOW METABOLISM COUPLING

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We have elsewhere in this meeting (Boutelle et al. [1]) used rapid sampling online microdialysis to measure the neurochemical signature of transient, spontaneous spreading depolarizations (SDs) detected in the injured human brain. It is not possible to decide from the measured electrocorticography signal alone whether the underlying neuronal tissue has a healthy response (increased blood flow) or the paradoxical fall in blood flow found in greatly at risk-tissue. To resolve this issue, a series of in vivo experiments was carried out in collaboration with the Max Plank Institute for neurophysiology, Cologne.

The experiments were carried out in the anaesthetized cortex. Local intracortical DC potential and extracellular [K⁺] levels were recorded using twin barrel ion-selective microelectrodes. A microdialysis probe was (Microbiotech) placed in the cortex and perfused at 1 mL min⁻¹. The dialysate stream was analyzed online for glucose and lactate using flow injection amperometric biosensor and for dialysate potassium using an online flow cell. Finally, cerebral blood flow was imaged quantitatively across the cortical surface using laser speckle imaging (Strong et al. [2]).

SDs were initiated in otherwise healthy tissue by microinjection of KCl and characterized using the microelectrodes and blood flow imaging as they tracked across the cortical surface. Comparison of tissue responses distant from the

microdialysis probe, adjacent to the probe, and following the probe showed no significant difference. Analysis of the dialysate stream showed a $[K^+]$ transient increase that coincided with the arrival of the increase in blood flow and the $[K^+]$ change seen in the tissue. This depolarization was associated with a transient increase in dialysate lactate level and a fall in dialysate glucose levels that had not been resolved within 30 minutes. This suggests that despite a 100% increase in blood flow, the metabolic demands of the SD overwhelmed the local glucose supply in even otherwise healthy tissue. The prolonged depression of glucose levels suggests that if such an event repeated, as often happens clinically, it could compromise tissue viability.

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DEVELOPMENT, VALIDATION, AND QUANTIFICATION OF PTERIDINE LEVELS IN PLANT SAMPLES BY HIGH-PERFORMANCE CAPILLARY ELECTROPHORESIS WITH LASER-INDUCED FLUORESCENCE DETECTION

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Plants are a primary source of folates in the human diet while pteridines are vital precursors for folate synthesis. Although Kohashi has analyzed for the presence of pteridines in a limited number of plants using high-performance liquid chromatography (HPLC), thin layer chromatography (TLC), and fluorescence analysis, very little is known about the levels, structures, and pathways of synthesis and turnover of pteridines in plants. Recently, Hossain, Schubert, and colleagues [PNAS 101:5158–5163(2004)] increased pteridine levels in plants 1200-fold through metabolic engineering. The increase in pteridine synthesis resulted in a corresponding enhancement in total folates in these plants. In this study, the presence and accumulation of a number of known and unidentified pteridines were reported. In order to understand the relationships between these pteridines and their role in plant metabolism, it is crucial to identify and measure the levels of the pteridines that accumulate in these genetically modified plants. Capillary electrophoresis (CE) offers an alternative and complementary method to HPLC and TLC to identify and quantify pteridines in plants. In an effort to provide a rapid and sensitive complementary technique, we have developed a method using capillary electrophoresis combined with laser-induced fluorescence (LIF)

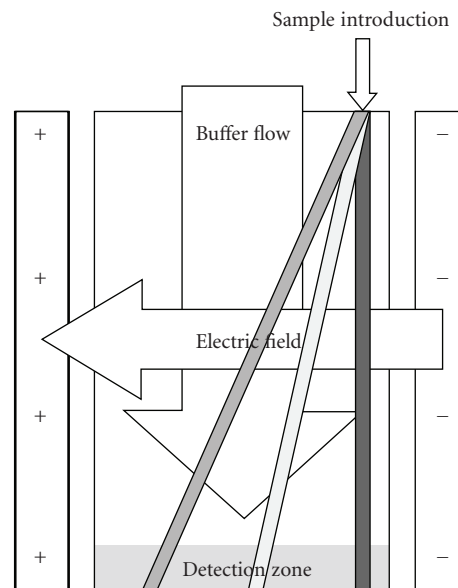


FIGURE 15

to identify and quantify the pteridine levels in plant samples of wild-type nontransgenic and transgenic plants engineered for increased pteridine synthesis. Specifically, nine pteridine derivatives were analyzed: 6,7-dimethylpterin, 6-biopterin, D-(+)-neopterin, 6-hydroxymethylpterin, pterin, monapterin, xanthopterin, isoxanthopterin, and pterin-6-carboxylic acid. The detailed information on the experimental conditions (such as buffer strength and pH, injection parameters, operating potentials, and wavelengths of excitation and emission), limits of detection, linearity, and methods of statistical analysis will be described.

FABRICATION, OPTIMIZATION, AND APPLICATION OF A MICRO-FREE FLOW ELECTROPHORESIS MICROFLUIDIC CHIP

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Micro-free flow electrophoresis (μ -FFE) is an analytical separation technique that continuously separates analytes based on electrophoretic mobility differences as illustrated in Figure 15. A $70\ \mu\text{m}$ wide channel allows for continuous injection of sample into a 1 cm wide channel pumped with separation buffer, creating a single sample stream under laminar flow conditions. An electric field is applied laterally to separate the single stream into the multiple components. The microfluidic chip was fabricated using anodic bonding of two etched and electrode-deposited wafers. To eliminate electrolysis product formation during separations, four-fold deeper channels were etched for the electrodes to facilitate a 16-time greater linear velocity. The removal of electrolysis products increased resolution of fluorescent standards by a factor of 1.3 under similar separation conditions.

Also, the new design allowed for a four-fold increase in applied electric field to 586 V/cm before joule heating conditions persisted. Bandwidths of a set of fluorescent standards were measured under different experimental conditions to determine the main cause of band broadening. A migration distance squared dependent mechanism was identified under nondiffusion limited conditions. Considering this, minimization of migration distance, possibly through suppression or modification of electroosmotic flow, allows for the highest resolution of analytes and efficiency of separations. Also, fundamental equations were derived to describe total peak variance, plate height and number, and resolution. Linear velocity emerged as an important variable for optimization. In addition, analyte streams can be precisely positioned in the separation channel based on linear velocity, total mobility, and applied electric field. Separations of biomolecules have been performed to show the improved functionality of the μ -FFE through knowledge gained from the fundamental studies.

NEW SAMPLE PREPARATION AND DATA ANALYSIS METHOD FOR ANALYZING 260 PESTICIDES

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Three matrices (tomatoes, green salad, lemons) were used in the study. All matrices were spiked at 4 levels (0.01–0.1 mg/kg) with a total of around 260 pesticides. Extraction and cleanup were done with the QuEChERS¹ method according to Anastassiades et al. [1] and determination with the GC/MS in scan and SIM modes. Method validation will be performed and discussed.

Reviewing of full scan GC/MS data for pesticide residue confirmation can be a labor-intensive and time-consuming process which requires concentration and experience. A new deconvolution software is able to process a complex food extract total ion chromatogram (TIC) in the order of 1-2 minutes, whereas an experienced analyst may take more than 10 minutes to achieve the same quality of results.

The deconvolution was done using AMDIS software (developed by NIST). AMDIS looks at every ion's apex and rising and falling patterns (peak width) throughout the TIC to extract related ions together into a deconvoluted spectrum. The matrix background or interference ions would then be left out of the deconvoluted spectrum. Each deconvoluted (cleaned) spectrum is then searched against a target library for hits.

Previous work was based on the sample preparation method according to Specht and Tillkes (modified DFG S19 method, ASU L 00.00-34) [2] and the result screener for data analysis. This presentation will compare the "old" and "new" approaches.

¹ (quick, easy, cheap, effective, rugged, and safe.)

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AUTOMATING THE DEVELOPMENT OF LIQUID CHROMATOGRAPHIC METHODS FOR IMPURITY AND STABILITY SAMPLES USING HIGH-SENSITIVITY, HIGH-RESOLUTION LC/MS

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Samples generated in support of purity and stability studies can be very complex. Closely related substances, disparate levels, and unknown analytes often present at low levels (0.1 to 0.05% or less) provide significant challenges for method development. In addition, as selectivity is manipulated and elution orders change, these same challenges make it even more difficult to track or identify peaks in the final method. This situation is particularly troublesome when trying to automate method development. Methods must also be robust, especially in the case of long-term stability studies, as they may be in use for long periods of time.

In this presentation, we will demonstrate a new way of completely automating the development of chromatographic methods for samples generated in support of purity and stability studies by employing new software algorithms and mass spectrometry (MS). Software tools will be described that fully automate column and method selection, while using MS to track and identify individual analytes, even at the low and/or disparate levels required. In addition, we will also illustrate a new way of developing methods from composite samples generated under multiple forced degradation conditions. Gains in sensitivity, resolution, and separation speed made possible by the use of sub-two micron particle size column chemistries will also be reported. Using this system approach, high-sensitivity, high-resolution methods can be developed in a fraction of time compared to traditional techniques.

ONLINE ELECTROCHEMISTRY/LIQUID CHROMATOGRAPHY/MASS SPECTROMETRY (EC/LC/MS) FOR THE SIMULATION OF THE OXIDATIVE METABOLISM OF PHARMACEUTICALS

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The online combination of electrochemistry, liquid chromatography, and mass spectrometry (EC/LC/MS) allows to

mimick the cytochrome P450 mediated oxidative metabolism of pharmaceuticals in the body. In a large volume flow-through electrochemical cell, which allows for the quantitative conversion of the target compounds under optimum conditions, drugs can be oxidized to their phase I metabolites. Further reactions, especially with scavenging compounds like thiols, can lead to the respective phase II products. The phase I and phase II metabolites can be separated and further characterized by online LC/MS.

The detoxification mechanism of paracetamol in the body was successfully mimicked using this online EC/LC/MS combination. Paracetamol was oxidized at a porous glassy carbon working electrode at a potential of 600 mV versus Pd/H₂ under formation of the intermediate N-acetyl benzoquinoneimine. In a subsequent reaction, the quinoneimine was quenched by formation of isomeric adducts with glutathione or N-acetylcysteine via the thiol function. The adducts were characterized online by liquid chromatography/mass spectrometry. The observed reactions were similar to those observed under catalysis of cytochrome P450 enzymes in the body. The formation of the thiol adducts was studied in absence and in presence of other thiol compounds that competed for the reaction with the quinoneimine. Although there are differences observed between kind and abundance of metabolites in the body and in EC/LC/MS experiments, it is evident that the technique may become a very valuable tool for pharmacological studies of new drug candidates.

MODELING OF DOPAMINE UPTAKE IN MURINE BRAIN SLICES USING AMPEROMETRY AND FAST-SCAN CYCLIC VOLTAMMETRY: A COMBINED TECHNIQUE

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Fast-scan cyclic voltammetry (FSCV) and amperometry at carbon fiber microelectrodes have been used for electrochemical measurement of dopamine (DA) dynamics in brain tissue. Each technique has expressed advantages and disadvantages. Amperometry has a fast temporal response because it immediately oxidizes molecules that contact the surface. The amperometry response therefore gives an accurate representation of DA dynamics. However, it lacks the selectivity achieved with FSCV. The temporal response of FSCV is limited by the rate of adsorption of DA to the electrode which must be removed by deconvolution techniques. Temporal resolution becomes especially important in transgenic animal models with faster than normal DA uptake. In these animals, it is possible that the rate constant of uptake will match or exceed that of the adsorption rate constant. A combined approach using FSCV and amperometry for measurement of release and uptake dynamics will allow for complete characterization of animals with faster than normal uptake. Acutely prepared murine brain slices from wild-type mice and mice expressing increased numbers of dopamine transporters (DATs) were characterized with FSCV and amper-

ometry. The temporal response was compared for both techniques; amperometric traces showed a response more rapid than FSCV. Dopamine uptake in transgenic mice was evaluated using FSCV and pharmacological manipulation. Amperometry was explored as a second way to verify uptake rate constants obtained with FSCV. Amperometry will serve as an important supplement in systems where uptake exceeds the temporal response of FSCV.

RAPID MONITORING OF ADENOSINE CONCENTRATION BY FAST-SCAN CYCLIC VOLTAMMETRY

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Adenosine is an important neuromodulator that regulates cerebral blood flow, modulates neurotransmission, and may be neuroprotective during stroke. Direct measurements of adenosine have been made using microdialysis sampling, but the temporal resolution is poor (usually 10 minutes), and the probe may damage blood-brain barrier. Carbon-fiber microelectrodes are frequently used as chemical sensors in biological preparations because of their small size and fast electron transfer kinetics. The goal of this study was to develop a method to determine physiological concentrations of adenosine at carbon-fiber microelectrodes using fast-scan cyclic voltammetry. A waveform for detection was optimized. The potential was scanned from -0.4 to 1.5 V and back at 400 V/s, sufficient to oxidize adenosine. Two oxidation peaks were detected for adenosine with T-650 carbon fibers. The second oxidation peak at 1.0 V occurs after the initial oxidation at 1.5 V and is due to a sequential oxidation step. The mechanism of the two oxidation peaks was studied by varying the switching potential and observing cyclic voltammograms taken directly after introduction of adenosine in the flow cell. The limits of detection were 15 nM, less than the 50–200 nM estimates of basal levels. The scan rate was varied and the kinetics were adsorption-controlled. The linear range of concentration detection was 200 nM to 20 μ M. Possible biological interferences were studied. The electrode was 3 to 5 times more sensitive for adenosine than AMP and ATP because of the negative holding potential. Guanine had an oxidation potential of 1.1 V and could be discriminated from adenosine in mixtures. Inosine was not detected at these potentials. These studies demonstrate that carbon-fiber microelectrodes can be used to detect physiological concentrations of adenosine and show promise for use as *in vivo* adenosine sensors.

COMPARISON OF MULTIVARIATE CALIBRATION MODELS FOR GLUCOSE IN BOVINE BLOOD ULTRAFILTRATE FROM NEAR INFRARED SINGLE-BEAM AND ABSORBANCE SPECTRA

Min Ren

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Near infrared (NIR) spectroscopy offers a painless and convenient method for measuring glucose concentrations non-invasively, thereby enhancing frequent monitoring and delaying medical complications of diabetes. Compared with single-beam spectra, absorbance spectra generally facilitate the development of linear calibration models and remove nonanalyte spectral variance if background spectra provide good match to sample matrix. However, the mismatch between sample and background spectra can be so severe in complex matrix like blood that it seriously degrades analytical performance. The work presented here demonstrates the potential of ultrafiltrate glucose measurements by analyzing samples collected from bovine blood, and further compares the single-beam and absorbance spectra on quantification ability.

Blood samples were obtained from ten cows from a local abattoir immediately after each animal was sacrificed. The blood was stored with EDTA as an anticoagulant and then passed through a KrosFlo hollow fiber ultrafiltration module coupled with a Materflex peristaltic pump. In all, 100 standard solutions were prepared from the collected ultrafiltrate and these samples were prepared with randomized concentrations of glucose, urea, and triacetin. NIR spectra were collected over the combination spectral region of the near infrared spectrum with Nicolet Nexus 670 Fourier transform spectrometer. Both single-beam and absorbance spectra were used to build partial least-square (PLS) calibration models for glucose and urea. Results demonstrate excellent calibration performance for measuring glucose and urea from single-beam and absorbance spectra. Correlations between pure component spectra and the corresponding PLS regression vectors indicate that the calibration models built from single-beam and absorbance spectra are based specifically on glucose spectral information. Furthermore, a pure component selectivity analysis confirms the selectivity of these calibration models.

NIR PROCESS MONITORING OF DILUTED MULTICOMPONENT AQUEOUS SOLUTIONS

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NIR process monitoring is a proven technology widely used in multiple industries. The most successful applications are related to moisture analysis, as well as analysis of concentrated organic materials in solid form or nonaqueous liquids. NIR analysis of diluted multicomponent aqueous solutions presents multiple challenges.

- (i) Spectra are dominated by water features.
- (ii) Analyzed components do not have their own spectral features and provide small shifts of water spectra.
- (iii) Shifts of water spectra due to chemical changes are very similar and typically smaller in magnitude than shifts related to temperature changes.
- (iv) Effects for dilute solutions are usually on the noise level of commercial instruments.

This presentation will describe multiple application of NIR for closed loop control of blending and wet processing operations in semiconductor industry.

REAL-TIME CONTROL OF MICROREACTORS BY RAMAN SPECTROSCOPY

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For more than half a century, the chemical manufacturing industry has produced commodity chemicals by scaling up 1-liter laboratory syntheses to million gallons per year productions. In the past few years, a new approach to chemical manufacturing is emerging, that is numbering-up. This new approach is based on two recent developments, parallel synthesis employed in combinatorial chemistry, and small-scale reactors that utilize the high surface-to-volume ratio at the nano- and microscales to improve chemical reactivity and selectivity. Many researches have shown that microreactors offer a number of advantages over traditional large-scale reactors, principally increased control of reaction pathways and hence product choice and yield. This is particularly true in the pharmaceutical industry where only small-scale synthesis is required until clinical trials are complete; at which time full-scale production needs to be accomplished in the shortest possible period. One of the most often used reaction steps during the synthesis of pharmaceuticals is protecting carboxylic acid groups by esterification. We have been developing Raman spectroscopy as a novel analytical tool to monitor and control chemistry in such small-scale reactors. Here, we will present Raman spectra of the esterification of benzoic acid, an important pharmaceutical reaction performed in a 5 mL continuous loop reactor, as well as rapid formulation development of a specialty sol-gel/polyethylene fiber.

AUTOMATED CONCENTRATION AND QUANTITATIVE TRANSFER TO REDUCE SAMPLE HANDLING

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Many sample preparatory processes involve concentration at one or more steps. Traditional concentration systems vary in their amount of automation and final volume precision.

A new concentration system offers a flexible platform to meet the varied concentration needs of laboratories. The system can be programmed to process up to 72 samples without user intervention. Sample volumes from hundreds to a few milliliters can be concentrated. Level sensors are utilized to constantly control the sample volume during processing. Flexible endpoint and quantitation options allow the user to program the system based on the target analyte list. The sample is then transferred to a vial ready for analysis. Programmable rinses are performed prior to the next sample.

This presentation shows the ability of a single concentration system to conduct multiple steps and decrease sample handling.

LAB-ON-A-CHIP SENSORS WITH HAND-HELD INSTRUMENTATION FOR QUALITY CONTROL OF DRINKING WATER IN MUNICIPAL DISTRIBUTION SYSTEMS: MANAGING CHLORAMINATION AND PREVENTION OF NITRIFICATION

Glenn B. Martin

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Insuring the quality of our drinking water is becoming an increasingly important and challenging mandate for municipal water plant operators. Among today's most pressing issues are

- meeting tighter regulatory limits for disinfection by products (e.g., carcinogens);
- making and managing the shift from free chlorine to chloramination;
- collecting a more detailed analytical profile of the distribution system;
- managing and acting on real-time field data.

To help meet these challenges, we have developed an electrochemical lab-on-a-chip sensor technology and associated instrumentation. This technology, consisting of an array of potentiometric, amperometric, and conductance sensors, delivers a water profile with 8 measured parameters and 6 calculated results in less than 5 minutes. Included in the analysis are pH, calcium hardness, carbon dioxide, free chlorine, total chlorine, ammonia, conductivity, and ORP.

An important application of this technology is monitoring the parameters necessary to prevent nitrification. The use of chloramination, chlorine combined with ammonia, is increasingly being used as the residual disinfectant. A difficulty with the use of monochloramine is that it decomposes, as it passes through the distribution system, thereby liberating ammonia. The loss of monochloramine along with increasing ammonia levels produces conditions which are conducive to the microbial growth. In a process known as nitrification, these microbes convert ammonia to nitrate. Once nitrification begins, microbial growth accelerates rapidly, but with frequent monitoring for key parameters, operators can adjust water chemistry appropriately, thereby preventing nitrification.

NEW AUTOMATED ATOMIC FORCE MICROSCOPE: THE NANOSURF NANITE AFM

Sebastian Kossek and Robert Sum

Nanoscience Instruments, 9831 S. 51st Street, Suite C119, Phoenix, AZ 85044, USA

Nanoscience Instruments announces the release of the Nanosurf Nanite automated AFM. Swiss-based Nanosurf AG, innovators and makers of the most compact and easy-to-use

atomic force microscopes (AFMs) on the market, have applied their inventive designs to the challenge of automated multiple measurements—the result is a new easy-to-use AFM that gives you true walk away from time. The Nanite's batch programming, scripting capability, and motorized X/Y/Z stage make it possible for users to prepare a series of routine measurements and leave the microscope to measure on its own. So, whether your process requires nanoscale measurements at random points on a large surface, or on multiple samples in a reproducible location, the batch-programmable Nanite AFM is up to the task.

The Nanite's patented electromagnetic scanners guarantee precise and highly linear movement for reliable quality-control measurements and statistical analysis. The integrated Nanosurf report software aids in quick, efficient evaluation and comparison of roughness, particle count and size distribution, and step heights. Typical applications range from coatings, polymers, fabrics, and fibers to semiconductors, wafers, optical, and holographic surfaces as well as data storage.

The Nanite AFM has a flexible adaptive design. Its scan head incorporates a highly accurate, quick-release mount for easy adaptation to other surface analysis instruments.

As with all Nanosurf AFMs, the Nanite features fast and simple AFM probe exchange due to prealigned optics that completely eliminate the need for manual laser or detector alignment, drastically reducing setup time. And you will appreciate the Nanite's on-board video camera which observes the AFM cantilever and sample from above and from the side, making probe positioning easy.

We invite you to experience for yourself the high precision and user friendliness of the new automated, batch-programmable Nanosurf Nanite AFM.

A NEW FULLY AUTOMATED POLYMER VISCOMETER DESIGNED TO COMPLETELY AUTOMATE THE MANUAL STEPS IN SOLUTION VISCOSITY TESTING

Lucy Innes

RHEOTEK USA, 1710 Mahogany Run Drive, Lagrange, KY 40031, USA

RHEOTEK USA presents a breakthrough in polymer viscosity testing. Current manual solution viscosity measurements, with their inherent inaccuracies and variability, often lead to costly retests and production delays when laboratory data and manufacturing data differ. RHEOTEK USA, in conjunction with ISO 17025 accredited laboratory Poulten Selfe and Lee Ltd., has identified the common variability in solution viscosity test methods. RHEOTEK has developed a robust, fully automated, laboratory practice to measure solution viscosity (IV) correctly at the first time, and every time.

Together, we will journey through a case study concerning the ASTM D 4603 method determination of IV of poly(ethylene terephthalate). Our discussion will include sample preparation using weight-to-volume versus

weight-to-weight methods, sample dissolution time versus temperature, low molecular weight polymers versus high molecular weight polymers, surface area of the polymer samples, and insoluble matter. We will also discuss how to design in-house studies to determine the variability of the viscosity measurement, the variability of the sample preparation, and the variability of the sample itself and how our automated polymer viscometer can solve your current and future solution viscosity testing needs.

ADVANCED LABORATORY SAMPLE DELIVERY AUTOMATION WITH RFID

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While vast improvements continue to be made in the areas of laboratory automation, procedures, and workflow, improvements in efficiency and quality seem to be occurring in smaller and smaller increments. It is as if there were a glass ceiling beyond which no lab can improve. The good news, however, is that this is a false ceiling. What is lacking is an innovative approach and perspective on laboratory automation and sample delivery within the lab. By combining a fresh approach with the correct technologies, such as RFID, laboratories can again make procedural and automation improvements that will bring both significant advancements in efficiency and impressive enhancements in quality to the laboratory.

NEW AUTOMATED KARL FISCHER EVAPORATOR SYSTEMS

John MacFarlane, Hajime Takahashi, Kouji Yamato, and Momoko Nagaya

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Karl Fischer titration remains one of the most widely used methods for measuring moisture across many industries. Despite the high selectivity of Karl Fischer (KF) reagents toward water, there are several compounds that react with KF reagents besides water. Evaporators or ovens are typically used to overcome these shortcomings as well as analyzing difficult samples such as various solids, powders, polymers, greases, and fatty products. However, such evaporators can only be used manually and thus they are tedious and time-consuming. For testing labs with heavy sample workloads, an automated KF-evaporator system would be extremely useful to maximize sample throughput.

In this report, we describe the improvement in the automation of the KF analysis of samples that are both difficult to analyze along with those with interfering substances. Data from a wide range of samples will be presented.

ONLINE MICRODIALYSIS WITH THREE-MINUTE TIME RESOLUTION USING AN EC DETECTOR WITH QUADRUPLE CELL CONTROL

Hendrik-Jan Brouwer, Lusi van Heerwaarden, Martin Eysberg, and Nico Reinhoud

Antec Leyden, Industrieweg 12, Zoeterwoude, ZH 2382Nv, Netherlands

A method has been developed for improved time resolution in online microdialysis shown in Figure 16. The method was developed for analyzing responses such as neurotransmitter release that take place within 30 minutes. Conventional microdialysis sampling once every 10 or 20 minutes is not enough to describe the release accurately. Analysis of dopamine (DA) and serotonin (5-HT) with 3-minute time resolution has been achieved. The HPLC system can also be used for analysis of noradrenalin (NA) together with DA and 5-HT using 2 columns with different mobile phase for the analysis of NA. The same hardware can also be used for analysis of microdialysates from 3 or 4 animals in parallel. Because only one detector and one pump are used, the system uses a small footprint. Detection limits improve with higher injection volume and they are between 20–100 pmole/L for, respectively, 10–2 μ L injections.

MULTICHANNEL ONLINE MICRODIALYSIS USING AN EC DETECTOR WITH TRIPLE CELL CONTROL

Hendrik-Jan Brouwer, Lusi van Heerwaarden, Martin Eysberg, and Nico Reinhoud

Antec Leyden, Industrieweg 12, Zoeterwoude, ZH 2382Nv, Netherlands

A method has been developed for multichannel online microdialysis, which is particularly useful when experiments must be replicated for statistical relevance. The method uses three HPLC columns in parallel integrated in one DECADE II electrochemical detector with triple cell control (TCC). An example is shown for analysis of serotonin in an online microdialysis simulation using dialysis probes (Figure 17).

OLFACTORY NUISANCE MONITORING WITH RQ BOX WIRELESS DEVICES NETWORK

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Purpose

In order to assess olfactory nuisances produced by industrial plants (sewage, composting, landfill, petrochemical plants, livestock carcass-quarantining sites), reference methods often involve human olfaction.

The electronic nose is a well-established instrument in the laboratory setting or in quality control where it is used to perform various volatile organic compound (VOC) or aroma analysis.

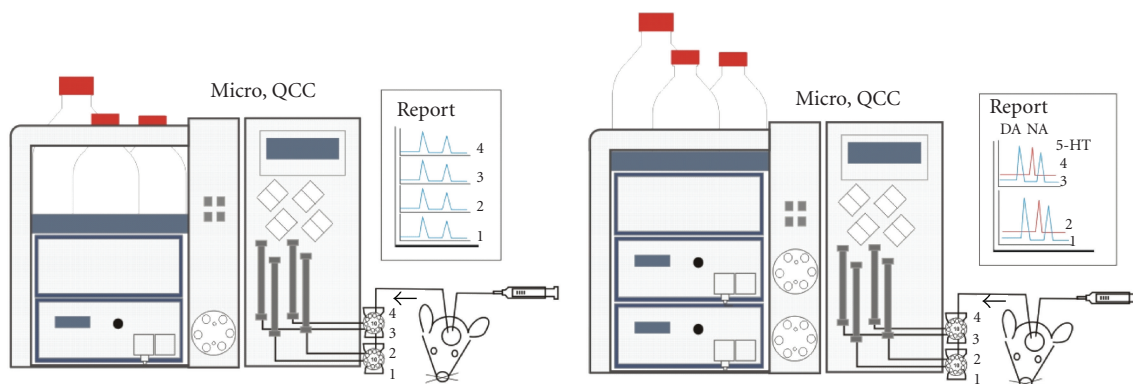


FIGURE 16: Configurations for parallel analysis. The system on the left is designed for analyzing NA and DA (or DA and 5-HT). The system on the right is designed for analysis of NA, DA, and 5-HT. In that case, two columns are used for NA/DA, and the other two for DA/5-HT.

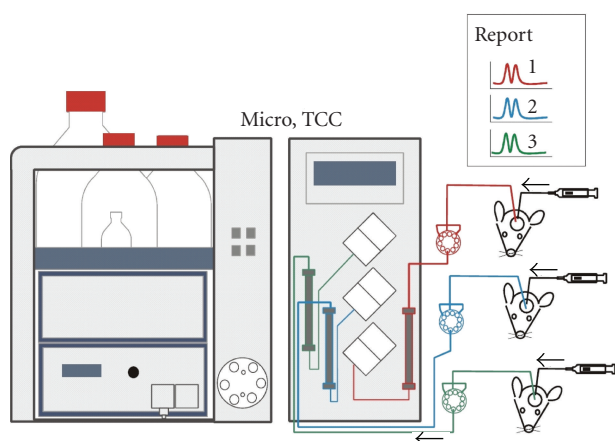


FIGURE 17: Samples from three online microdialysis experiments are injected simultaneously and analysed in parallel.

In order to offer continuous and real-time monitoring of olfactory nuisances, specific sensor array devices have been developed. This small-sized equipment (RQ box as air quality box) includes several complementary sensor arrays in order to detect volatile chemicals, solvents, and odors with dedicated databases.

Method

The network installed in a leading European landfill company will be presented. The results correlated with the standardized method (EN 13725) will be detailed.

Conclusion

This new generation of electronic nose devices dedicated to environmental olfactory nuisances is efficiently used for improving the quality and safety of industrial VOC emissions.

ACKNOWLEDGMENT

Thanks to real-time measurement ability and wireless communication, these devices can be installed in several areas of

the same industrial site and generate an accurate map of olfactory nuisance.

FIELD TRIAL RESULTS OF A MULTISPECIES ANALYZER FOR MONITORING ATMOSPHERIC LEVELS OF METHANE AND CARBON DIOXIDE

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Methane (CH_4), water vapor (H_2O), and carbon dioxide (CO_2) are collectively responsible for the majority of the earth's greenhouse effects. Robust instrumentation that could measure these gases with both high accuracy and high precision would reduce the uncertainty in the determination of terrestrial sources and sinks of these dominant greenhouse gases, resulting in improved predictive models and a better understanding of the human contribution to global warming.

Current atmospheric measurement instrumentation has been exposed to problems related to the dependence of its readings on operating temperature and operating pressure. Many need frequent zero and span calibrations, which requires keeping and maintaining gas cylinders with zero air and standard gas mixtures for CO_2 , and dew point generators for water at each measurement site. In addition, many cannot easily be simultaneously calibrated from site to site to the level of accuracy required for use in atmospheric inversion studies.

In this paper, we present results from field trials of a newly available analyzer, based on cavity ring-down technology, capable of performing, in a single instrument, atmospheric inversion measurements of CO_2 , CH_4 , and H_2O . The instrument is designed such that it does not require frequent calibration and maintains high linearity, precision, and accuracy for long periods of time, over changing environmental conditions, with little or no sample preparation. Deployment of this analyzer could reduce operating costs and greatly improve the accuracy of greenhouse gas measurements, worldwide.

ARSENIC SPECIATION IN ATMOSPHERIC TOTAL SUSPENDED PARTICLES USING ATOMIC FLUORESCENCE SPECTROMETRY

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An arsenic speciation study was performed using air filters on which total suspended particles (TSPs) were collected from the city of Huelva, a medium size city with huge industrial influence in SW Spain. Different extraction procedures were performed using water, $\text{NH}_2\text{OH}\cdot\text{HCl}$ and H_3PO_4 solutions with either microwave or ultrasonic radiation. The optimal extraction procedures were found to be with the use of $100 \text{ mmol L}^{-1}\text{NH}_2\text{OH}\cdot\text{HCl}$ and $10 \text{ mmol L}^{-1}\text{H}_3\text{PO}_4$ with microwave radiation for 4 minutes. High-performance liquid chromatography coupled with hydride generation and atomic fluorescence spectrometry (HPLC-HG-AFS) was employed for the determination of arsenic species. The average extraction efficiency for air filters collected on a monthly basis ($n = 12$) was found to be 94% and 86% with $\text{NH}_2\text{OH}\cdot\text{HCl}$ and H_3PO_4 , respectively. Only inorganic arsenic species (arsenite and arsenate) were detected. The annual mean arsenite and arsenate concentrations were found to be $1.2 \pm 0.3 \text{ ngm}^{-3}$ and $10.4 \pm 1.8 \text{ ngm}^{-3}$, respectively. Arsenate levels were found to be more variable with maximum concentrations reaching 30.6 ngm^{-3} . The high level of arsenic species in the TSP samples can be related to a copper smelter located in the region.

MATHEMATICAL MODELING OF CHRONOPOTENTIOMETRY FOR ION-SELECTIVE MEMBRANES

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The detection limit of most ion-selective electrodes (ISEs) is around 10^{-6} M . Recently, it was discovered that the detection limit is controlled by minor ionic fluxes, that is, leaching of ions from the membrane into the sample solution. This leaching increases the ion concentration at the surface of the sensing membrane compared to the sample bulk. Among other approaches, the application of a small galvanostatic current, which eliminates the flux of ions into the sample, has been used to prevent ion leaching and improve the detection limit of ISEs. In this work, a mathematical model is presented to describe the effect of a constant current on ion-selective membranes. From this model, we can predict the time course of the membrane potential and the electric field inside the membrane following the application of the current. We can also predict how the ionophore and ionophore-ion complex concentrations vary with space and time within the membrane. In Figure 18, a set of concentration profiles are

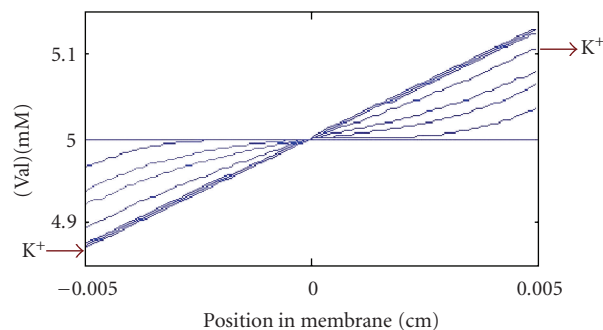


FIGURE 18: Valinomycin concentration profiles inside the membrane at several time instances.

shown, representing the time-dependent concentration polarization of the ionophore valinomycin in the cross-section of a 0.2 mm thick potassium-selective membrane upon the application of 5 nA/cm^2 current. To confirm the simulated concentration profiles, they are compared to experimental concentration profiles observed using spectra electrochemical microscopy.

Another application of these simulations is with larger currents used to characterize the membrane composition. The chronopotentiometric transients show a characteristic breakpoint when either the free ionophore or the ionophore complex reaches zero concentration at one of the phase boundaries. The model can predict when the breakpoint occurs and its dependence on the membrane composition and experimental conditions.

In conclusion, this model provides quantitative description of the membrane processes inside an ion-selective membrane when current is applied, and predicts the time required to obtain adequate voltage stability.

ACKNOWLEDGMENTS

This work has been supported by the NSF 0202207 and 0335228 Grants, and a grant from the NSF Graduate Research Fellowship program.

PROFILING PRIMARY FATTY ACID AMIDES IN MAMMALIAN TISSUE: LC/MS VERSUS GC/MS

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The amount of primary fatty acid amides (PFAMs) in a particular mammalian tissue can be determined by using liquid chromatography with atmospheric pressure chemical ionization mass spectrometry (LC/APCI-MS) for a secondary separation and analysis. Folch-Pi extraction done to remove PFAMs from tissue also removes other classes of neutral lipids. Because the raw extract is too complex to allow for trace analysis of PFAMs, the extraction is followed by solid phase extraction (SPE) to separate the PFAMs from the other classes. It is impossible to distinguish the position of a double

bond in the PFAMs by just doing one dimension of MS. So another separation is needed to individually separate the PFAMs before analysis. Standards are used to determine the elution order. Detection limits are around 20 fmol. For comparison, a gas chromatography mass spectrometry (GC/MS) method has previously been developed by Sultana with detection limits around 100 fmol. The sample preparation is the same for the GC method as the LC/MS except that an extra derivatization step is needed before analysis. A variety of mammalian tissues have been analyzed by both methods to determine which PFAMs are present in a variety of mammalian tissues.

ACKNOWLEDGMENT

This work is supported by NIH/NINDS.

APPLICATION OF MICROFABRICATION TECHNIQUES FOR THE DEVELOPMENT OF A MINIATURIZED ASV SYSTEM

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Anodic stripping voltammetry (ASV) is well established as a highly sensitive instrumental method ideally suited for heavy metal analysis. In this work, our goal has been the development of a miniaturized, battery-powered ASV device which could work unattended and determine, both qualitatively and quantitatively, heavy metals (e.g., Cd^{2+} , Cu^{2+} , Hg^{2+} , Pb^{2+}) in drinking and waste waters. In particular, we want to examine the use of modern microfabrication techniques in order to optimize the electrode design and performance for practical ASV applications.

Our prototype device consists of a flow-through system with a high-surface-area microfabricated electrode for metal preconcentration and downstream electrodes for detection. The preconcentrating electrode is fabricated by sputtering a gold film (tantalum underlayer for adhesion) onto an oxidized silicon wafer which is then patterned photolithographically to provide the desired shape and dimensions. Subsequently, 10–50 μm holes, in varying densities, are etched through the wafer by deep reactive ion etching in order to form a flow-through mesh. Downstream electrodes are created in a separate operation. Overall, this approach permits convenient control and variation of electrode area, shape and spacing, the size and density of flow-through channels, and other structural features to obtain optimum ASV performance. Although microfabrication procedures are best suited for the formation of Au or other metallic electrodes, postfabrication modification to more ideal ASV electrode materials such as Bi or boron-doped diamond is possible.

ACKNOWLEDGMENT

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DEVELOPMENT AND OPTIMIZATION OF NANOSCALE ARCHITECTURES FOR ENHANCED ELECTROANALYTICAL METHODS

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Miniaturization in the field of analytical chemistry continues to advance measurement capabilities in the biomedical sciences by enlightening our knowledge of complicated biological systems. Novel nanoelectrode fabrication technologies have been developed that will provide higher-spatial and faster-temporal measurements of small molecules associated with cellular function, specifically, those involved in neuronal communication. We have developed a method using focused ion beam (FIB) to mill through insulating layers of poly(methylmethacrylate) (PMMA) to fabricate nanoelectrode templates. FIB milling is a technique that provides precise spatial control of nanoscale fabrication. Such control allows for fabrication of single pore electrode templates as well as multiple pore array templates. Electroless decoration of milled PMMA with gold is used to fabricate several types of nanoelectrode elements such as inlaid disks, recessed wells, and free-standing wires. Enhancement of spatiotemporal resolution is a direct result of restricted diffusion and molecular confinement at nanoscale electrochemical elements. Pure radial diffusion at each electroactive element of fabricated arrays can be achieved by precise control of spacing, size, and density of nanopores milled in the array template. In situ characterization of these templates and electrode architectures is performed using SEM imaging. Nanoelectrode arrays (NEAs) and single nanoelectrodes are further studied using cyclic voltammetry and ultra sensitive optical microscopy. Preliminary cyclic voltammograms of single nanoelectrode template constructs have shown promise for electrochemical detection at such small scales (Figure 19). Optimization of this system lends itself to eventual integration into nanofluidic devices and coupling with lithographic techniques to increase spatial control for electrogenerated chemiluminescent events and single-cell handling and analysis.

DEVELOPMENT OF INTEGRATED CONTROL SYSTEM FOR MULTIPLE-SCANNING-PROBE MICROSCOPY

Higuchi Seiji, Komatsubara Takashi, Laurent Olivier, Nakayama Tomonobu, Obori Kenichi, and Yoshioka Seiichiro

Horiba Jobin Yvon, 2 Miyanohigashi, Kissoin, Minami-Ku, Kyoto 601-8510, Japan

Nanometer-scale electronic devices have been proposed and fabricated so far using various nanomaterials. For the characterization of signal transport (of electrons, ions, etc.) in such nanometer-scale devices, a multiple-scanning-probe microscope (MPSM) which has more than two independently

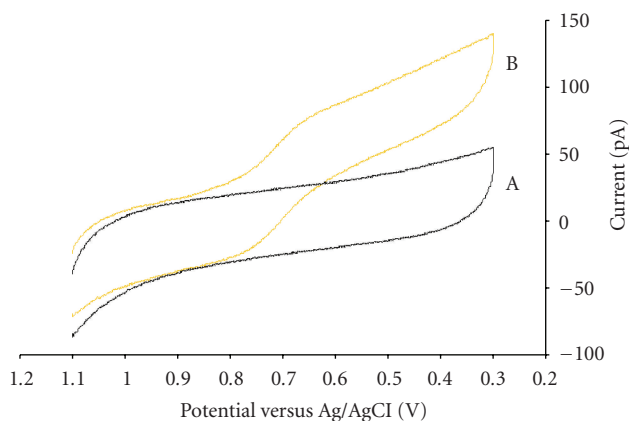


FIGURE 19: Cyclic voltammogram using ~ 55 nm rad. Single nanopore template: (A) 0.1 M potassium nitrate background scanned at 0.1 V/s; (B) 2 mM potassium hexachloroiridate in 0.1 M potassium nitrate scanned at 0.1 V/s.

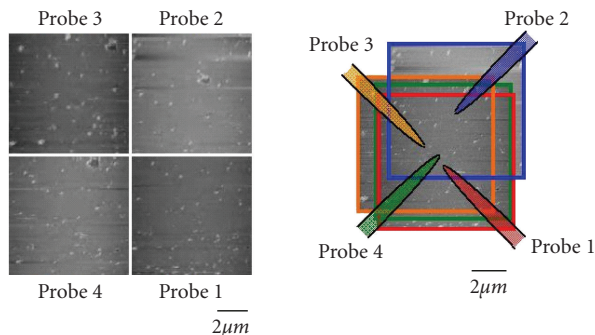


FIGURE 20: Simultaneously measured four STM images using four independent probes. The sample used here was the Pt/Si surface. White arrows indicate some of the characteristic features on the surface. $V_{tip} = -2.0$ V, $I_{ref} = 300$ pA, Scan area: 7800×7800 nm.

controlled probes would be a promising tool. In comparison with a normal SPM, the development of an MPSPM requires many additional functions. One of the important functions is to know the positional relationship between the probes on the nanometer scale. This function would be needed to avoid crashing into each probe and to find the nanostructure which is to be measured. For this purpose, several approaches have been reported so far. We have achieved simultaneous image acquisition by multiple probes and applied image recognition to automatically depict the overlapped areas between four images. A home-built quadruple-scanning-probe tunneling microscope (QSP-TM) which works in air is used for evaluation of the system.

In this presentation, we show recent development of our integrated control system which enables us to know the positional relationship between four probes on a sample surface with simple and easy operation. We have achieved simultaneous imaging on a Pt coated Si surface using multiple probes, and we have applied image recognition functions to automatically visualize the overlapped areas between each image (Figure 20).

PROCESS ANALYSIS WITH RAMAN SPECTROSCOPY: EXPANDING THE APPLICATION'S HORIZON

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The last 15 years have seen tremendous advances in the instrumentation for Raman spectroscopy. Three of these advances have been a reduction in the size of instrumentation, an increase in analyzer sensitivity, and the availability of fiber optic probes for remote analysis. These advances opened the door for process measurements using nondestructive, chemically specific, remote Raman spectroscopy. In this presentation, the next generation of developments in Raman analyzers will be discussed. These developments have expanded the applications space for Raman spectroscopy. Novel process monitoring and control applications opportunities relevant to the chemical, polymer, and pharmaceutical industries will be shown.

CHARACTERIZATION OF POLYCYCLIC AROMATIC HYDROCARBON DERIVATIVES USING LC/MS TECHNIQUES

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Polycyclic aromatic hydrocarbons (PAHs) are carcinogenic pollutants formed in incomplete combustion processes, including the burning of fossil fuels. They have been carefully monitored in environmental water, air, and soil samples for decades. Less attention has been paid to the characterization of oxidized PAH derivatives in the environment, primarily because standard analysis techniques do not have the required sensitivity and selectivity. This paper will present quantitative and sensitive liquid chromatography mass spectrometry (LC/MS) methods for the detection of benzo[a]pyrene quinones in environmental samples. It will discuss complimentary LC/MS/MS experiments that enhance the specificity of this analytical technique. Figure 21 presents the APCI-MS chromatograph of BaP-1,6-quinone, BaP-3,6-quinone, and BaP-6,12-quinone standards dissolved in methylene chloride obtained using a 70 : 30 methanol: water isocratic eluent transferred through the 30°C column at 1.100 mL/min. For this experiment, the APCI head operated in the positive ion mode at 500°C while applying 6.0 kV to the needle. Chemometrics were employed to determine the optimal operating conditions. The 3-sigma limit of detection of BaP-1,6-quinone is 4 ng/mL, and a linear calibration has been performed over four orders of magnitude. Results for the analysis of BaP-3,6-quinone and BaP-6,12-quinone are comparable. These techniques can be used to elucidate the primary mechanism of BaP photodegradation in complex media, and characterize BaP-quinones in environmental

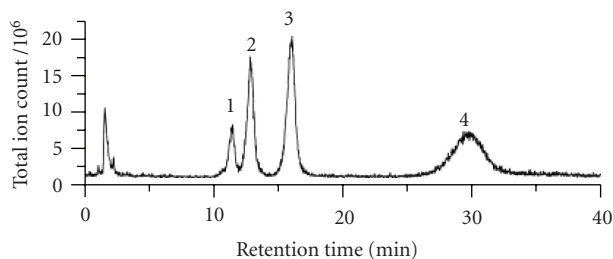


FIGURE 21: APCI-MS chromatograph of benzo[a]pyrene (BaP) and three benzo[a]pyrene quinones in a methylene chloride solution. Peak assignments are (1) BaP-1,6-quinone, (2) BaP-3,6-quinone, (3) BaP-6,12-quinone, and (4) benzo[a]pyrene.

samples. This study can also be used as a foundation for developing quantitative techniques for the characterization of not only other PAH degradation products, but also similar compounds of great significances to environmental, industrial, and pharmaceutical applications.

DEVELOPMENT AND APPLICATION OF QUANTITATIVE REAL-TIME FIELD-DEPLOYABLE AEROSOL MASS SPECTROMETERS FOR THE ANALYSIS OF SUBMICRON PARTICLES

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Aerosols (small particles suspended in air) play major roles in climate and the hydrological cycle, as well as on human health effects, visibility degradation, and deposition of acids, toxics, and nutrients to ecosystems. Aerosols are also used in material manufacturing, nanotechnology, and pharmaceutical drug delivery. The properties and effects of aerosols are strongly dependent on one extensive property (their concentration) and 3 intensive properties: size, chemical composition, and physical shape (or morphology). The measurement of atmospheric aerosols is difficult due to their inherent complexity, as well as to the large variability of their properties in space and time. The past decade has seen the emergence of several methods capable of determining the size and chemical composition of aerosol particles in real time using mass spectrometry. The aerodyne aerosol mass spectrometer (AMS) is currently the most widely used instrument of this type.

This presentation will focus on four new instrumental and data analysis techniques for aerosol field measurements with the AMS. (a) A high mass resolution aerosol mass spectrometer has been developed, which allows for the separation of fragments with the same integer mass. (b) A thermodenuder (TD) + AMS combination has been developed

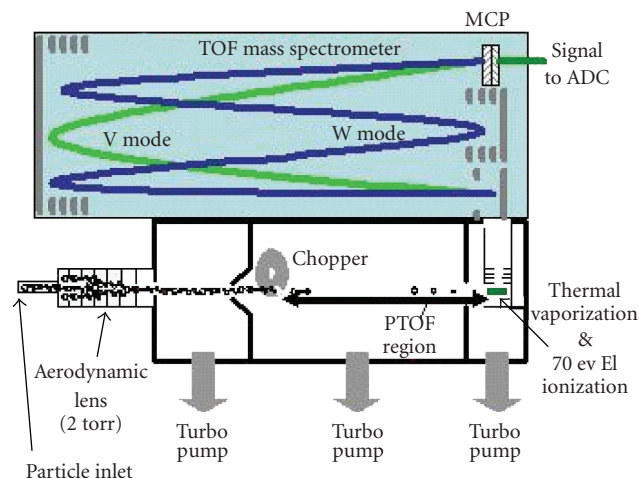


FIGURE 22

to obtain correlated volatility-chemistry determination. (c) A system for the measurement of chemically resolved eddy covariance aerosol flux measurements has been developed using the AMS. (d) A factor analysis technique has been developed for characterization of the organic aerosol components based on the entire organic mass, rather than on tracers (Figure 22). The basis of the technique and its application to the analysis of ambient particles at multiple urban, rural, and remote locations in the Northern Hemisphere will be discussed.

IDENTIFICATION OF IMPURITIES AND DEGRADATION PRODUCTS IN DRUG SUBSTANCES

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With advancement in ionization methods and instrumentation, mass spectrometry has become a powerful technology for the characterization of pharmaceuticals. This presentation will give an overview on approaches and strategies for the identification of unknowns in drug substances by mass spectrometry. Examples will include structural analysis of trace-level process impurities in steroid and other bulk drugs, and degradants identifications in degradation studies of drug substances.

AUTOMATION AND INFORMATICS TO PROFILE DEGRADATION PRODUCTS

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As lead compounds are progressed into a regulated manufacturing environment, analytical separations must evolve to accurately track the process. In addition to reactants and products, a strong drug substance method will also monitor the

degradation profile of the target compound. The generally accepted technique for determining the required resolution of a separation involves subjecting the target compound to aggressive environments to force the degradation of the compound. The process of generating degraded samples, reducing data, and collating results is time- and labor-intensive. We have implemented techniques and approaches aimed at softening the personnel resources dedicated to this work.

We will demonstrate an automated platform which allows for exposure of compounds in 96-well plates to acid, base, and temperature conditions. This platform allows for the rapid generation of samples in support of this work. The degradation methods are simple to implement and modify, allowing a wide variety of conditions to be examined.

Additional advances were made in the data analysis and interpretation of the separation data. Analysis tools are still in the early stages but promise great utility. Storage of conditions, retention times, and other useful data in a database allows for data-driven decisions around separation enhancement. The data also provide a backdrop of information relevant during method transfers to CMO/CROs or during process improvements. These reduce method development and qualification hurdles.

DETECTION AND MAPPING OF FINGERPRINTS BY MICROLIBS

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We are currently investigating the detection and mapping of latent fingerprints using micro laser-induced breakdown spectroscopy (MicroLIBS). MicroLIBS utilizes pulse energies of the order of 100 mJ or less in order to minimize damage to the surface under investigation and minimize crater size which in turn maximizes spatial resolution. Latent fingerprints which arise from the transfer of skin oils from a fingertip to a surface can be easily detected by the presence of sodium line emission from salt contained in the oil. LIBS offers an alternative to the traditional optical methods (typically fluorescent imaging) used to detect fingerprints as it directly detects the elemental signature of the latent fingerprint. Recent work by Lopez-Moreno et al. has demonstrated the possibility of detection of human fingerprints at a distance of 30 m using 350 mJ Nd:YAG laser pulses [1].

In the current report, the detection and detailed mapping of fingerprints by MicroLIBS are demonstrated using 80 microJoule 400 nm and 5 micorJoule 266 nm frequency converted Ti: Sapphire laser pulses. 2D imaging of regions of a fingerprint on a Si wafer is demonstrated. The imaging can be carried out by either looking at the sodium emission from the ridges of the fingerprint or the substrate emission signal, silicon in this case, from the valleys of the fingerprint. The thickness of the latent fingerprint ridges is found to be enough to suppress the line emission from the Si substrate when using low-energy femtosecond laser pulses. The emis-

sion scaling and optimum conditions for fingerprint detection are under study and will be reported.

ACKNOWLEDGMENTS

Funding of this research by MPB Technologies Inc. and the Natural Sciences and Engineering Research Council of Canada is gratefully acknowledged.

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SPECTRAL MODELING AS A MEANS OF OBTAINING CALIBRATION-FREE ANALYSIS IN LASER-INDUCED BREAKDOWN SPECTROSCOPY

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It has been demonstrated that within certain accuracy, it is possible to find the composition of a laser-induced plasma using computational methods and applying no calibration standards. A number of manuscripts dedicated to development of absolute analysis or so-called "calibration-free" methods have been published in the past several years. Similar to the previously reported works, the model we developed is based on the local thermal equilibrium (LTE) assumption and takes plasma temperature, plasma electron density, as well as the characteristics of atomic emission transitions as main input parameters. Using these parameters, we model the plasma emission spectra and predict the sample composition using a proposed calibration-free algorithm.

The model uses an atomic emission lines database to create a theoretical emission spectrum of selected elements for defined plasma parameters. The resulting theoretical spectrum is fitted to the experimental spectrum, which is obtained using the plasma emission signal collected by four separate compact spectrometers providing the spectral coverage of 185–950 nm. A calibrated light source is used to correct the experimental data for different spectrometer grating efficiencies, nonlinear CCD responses, and differences in the collecting optics. Elemental concentrations are obtained via the comparison of the observed and predicted spectra, the best fit being obtained by varying the essential plasma parameters.

SPECIATION ANALYSIS OF MERCURY IN FOOD SAMPLES BY GC-MIP-OES

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Plasmas are very efficient excitation sources for atomic spectroscopy. The microwave-induced plasma (MIP) sources

can be coupled to gas chromatography (GC) quite easily. While an inductively coupled plasma (ICP) consumes about 15 L/min of the noble gas argon, the MIP is operated with only 200 mL/min helium. Therefore, hyphenation of an MIP with a GC constitutes an efficient tool for speciation analysis.

The GC-MIP system presented allows for the quick analysis of mercury species in food samples. During sample preparation, mercury species are converted into volatile compounds via derivatization with NaBEt₄. The separation of the species is achieved with a 100% dimethyl polysiloxane GC column, followed by the detection in the MIP. The emitted light is selected by an interference filter which is oscillating in the light path between the plasma and the photo diode. Transmitting only a small section around the mercury emission line at 253.652 nm, this filter's main characteristic is that tilting it towards incoming radiation results in a shift of the transmission profile to lower wavelengths. With the oscillation of the filter and a modulated signal readout, a suitable background correction is possible.

The presented data show that selective and sensitive detection of mercury species in food samples can be realized with this system. The detection limit reaches down to 1 pg absolute in combination with a selectivity of mercury against carbon exceeding 10⁶.

AUTOMATED SPE FOR DRUGS IN BIOLOGICAL MATRICES USING A NEW 96-WELL POLYMERIC METHOD DEVELOPMENT PLATE AND AN OPTIMIZED PROTOCOL FOR FAST ROBUST METHOD DEVELOPMENT

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In spite of the versatility of a number of functionalized polymeric sorbents for furnishing clean extracts during solid phase extraction- (SPE-) based purification/enrichment of pharmaceuticals from plasma and other biological matrices in high-throughput environments, method development for sample preparation still retains a bottleneck. This is due to the tedious and time-consuming process of screening several sorbents individually, since very few formats with multiple sorbents packed in the same well plate are available. Additionally, each sorbent has its own optimized load, wash, and elution profile and not many reported procedures are available for using a common procedure for screening all sorbents.

In this presentation, we describe a protocol that can be used to screen multiple sorbents simultaneously, using a recently introduced 96-well method development plate. This new product is packed with four polymeric sorbents of divergent surface chemistries, namely, a neutral polar functionalized polymer strata-X, a strong cation exchanger strata-X-C, a weak cation exchanger strata-X-CW, and a weak anion exchanger strata-X-AW. All the sorbents are poly[stryrene-divinylbenzene]-based. A novel protocol is used to simultaneously evaluate all the sorbents under multiple conditions

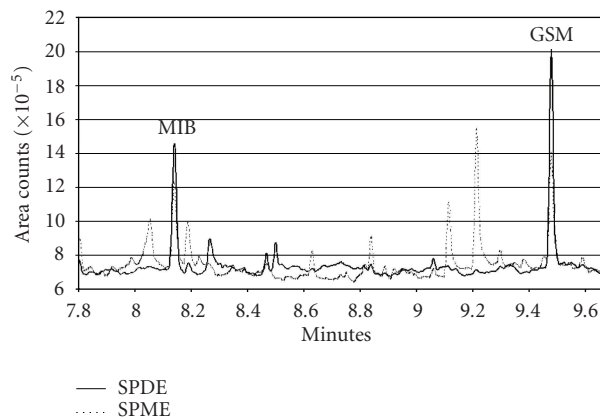


FIGURE 23: Total ion chromatograms of methylisoborneol (MIB) and geosmin (GSM) analyzed by SPME and SPDE.

and pinpoint the one set most suitable for a particular pharmaceutical compound. The protocol also enables the optimization of the load, wash and elution conditions for neutral, acidic, or basic analytes of a wide range of polarities. Several examples of the solid phase extraction of polar and nonpolar antibiotics, antihypertensives, nonsteroidal anti-inflammatory drugs, and antipsychotics from human plasma will be presented to illustrate the applicability of this rapid automated screening technique using MultiPROBE II liquid handling system.

COMPARISON OF SEVERAL METHODS FOR THE RAPID, AUTOMATED ANALYSIS OF FOOD FLAVORS

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Four rapid, automated methods are used to analyze volatile flavor compounds in a variety of food and food ingredients. The methods include membrane-assisted solvent extraction (MASE), solid phase microextraction (SPME), solid phase dynamic extraction (SPDE), and in-tube extraction (ITEX). Foods analyzed include rice, cantaloupe, honeydew melon, coffee, chocolate, pepper sauce, carbonated beverage, milk, orange juice, off-flavor sugar, and an artificial punch flavored beverage. A mixture of chemicals with a range of polarities is analyzed to demonstrate how different trapping materials are required to optimize the analysis of different materials.

All four methods are simple in terms of sample preparation. In all cases, samples are sealed in vials and loaded on an autosampler.

The limits of detection, coefficients of variation, cost per analysis, and linear range for each method are reported and related to the physics and chemistry of each method. For example, due to the larger amount of stationary phase in an SPDE needle as compared to an SPME fiber, SPDE traps more volatile compounds from the headspace (Figure 23) and therefore it has a lower limit of detection. Finally, pa-

rameters for each method are optimized for the analysis of each of the above-mentioned foods.

ACCELERATING THE DRUG DEVELOPMENT PROCESS VIA AN AUTOMATED SPE EMPLOYING A SHAPE-BASED NANOTECHNOLOGY AS A CHIRAL DISCRIMINATOR

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Enantiomers differ in three-dimensional conformation. The body can readily distinguish among isomers. Enantiomers can differ markedly in their pharmacokinetics, therapeutic efficiency, toxicology, and biological properties.

The FDA has actively encouraged the pharmaceutical industry to pursue enantiopure chiral drugs whenever possible. Significant time and resources are invested in their production. Current technology is costly and cumbersome as each chiral separation requires a great deal of process development. Single enantiomer drugs are so difficult to produce in quantity that only 500 exist out of the nearly 16 000 regulated pharmaceuticals. A unique window into chiral phenomena based on physical mechanisms rather than chemical interactions has been incorporated into a proprietary nanostructured "smart materials" providing unusual chiral properties. This revolutionary chiral medium based on physical chiral mechanisms offers a new and different approach to the challenges of chiral chromatography acting as a universal chiral selector. Incorporation of this unique chiral discriminator into solid phase extraction cartridges offers an extremely efficient and unprecedented performance. This dramatically reduces the time and cost of bringing new drugs to market. This simplistic approach offers the same separations observed in HPLC chiral chromatography and eliminates the requirements for a chiral detector. The automated solid phase extraction system allows multiple isomeric compounds to be separated in parallel at a fraction of time associated with analytical chiral chromatography. The "smart material" within an SPE cartridge is also being evaluated as a reusable medium which may offer an unprecedented approach to chiral separations.

METHOD DEVELOPMENT AND VALIDATION FOR DETERMINATION OF ADENOSINE IN BIOLOGICAL SAMPLES USING REVERSED-PHASE LIQUID CHROMATOGRAPHY

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Adenosine is a ubiquitous signaling nucleoside that is rapidly generated as a net result of ATP (adenosine triphosphate) catabolism that occurs in situations of cellular stress or damage. Once generated, adenosine can influence a diverse array of cellular functions by engaging cell-surface adenosine receptors. The expression of these receptors is widely distributed among tissues, and this signaling pathway has

been implicated to exert important physiological effects in the cardiovascular, neurological, renal, and immune systems. The complexity associated with adenosine metabolism and signaling makes the examination of its functions critical. Adenosine has been implicated to play a role in inflammatory lung disease such as asthma and chronic obstructive pulmonary disease. To investigate all of the above important roles of adenosine in the system, a reliable analytical method must be available to measure adenosine.

An effective HPLC method was developed and validated to determine adenosine in biological samples using Agilent 1100 HPLC system. A reversed-phase mode with a UV-detector at 254 nm and Supelco Discovery C18 column (4.6 × 250 mm, 5 m) was used. A gradient elution with a gradient time of 40 minutes and two segments, from 7–20% and 20–85% MeOH/buffer at pH 3.80 with 25 mM potassium dihydrogen phosphate, were found as the optimum conditions. These conditions resulted in a retention time of 13.3 minutes for adenosine.

The developed method showed a linear detector response with concentration of adenosine, with a correlation coefficient of 0.999 and a detection limit of 25 µg/L. Results of validation studies show that the developed method is specific, accurate, robust, and precise.

A CONTINUOUS FLUORESCENT ASSAY FOR PHOSPHOLIPASE C WITH CONJUGATED POLYELECTROLYTES

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A fluorescent turn-off assay for phospholipase C (PLC) from *Clostridium perfringens* is developed based on the traceable interaction between natural substrate, phosphatidylcholine, and PPE-type conjugated polymer with sulfate side group. The polymer fluorescence response to the effect of phosphatidylcholine is calibrated first and served as a basis to monitor the activity of the enzyme. The assay applies to micromolar regime of concentrations of substrate and offers a simple, fast, and reliable detection method to PLC with high sensitivity. Reaction rates and kinetic parameters are measured in real time by converting fluorescence signals of polymer to substrate concentrations. Activation and inhibition of enzyme activity are investigated. Good selectivity is obtained by testing the activity of PLC and five controls in the same assay, respectively, which demonstrates that the specific effect between substrate and enzyme is the dominant interaction.

A QUICK AND ACCURATE METHOD FOR DETERMINING MERCURY LEVELS IN FISH TISSUE UTILIZING DIRECT MERCURY ANALYSIS

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The determination of mercury in fresh- and saltwater species has become a primary focal point of both private and public

institutions. These organizations make important regulatory and scientific decisions dependent upon the mercury levels found in fish tissue and, therefore, require a rapid, accurate method for mercury determination. Traditionally, cold vapor atomic absorption spectrophotometry (CVAAS) has been used to measure mercury levels in fish. Although effective, this technique is a time- and labor-consuming method that relies on complicated wet chemistry sample preparation. In contrast, the mercury levels determined here were collected using Milestone Inc.'s DMA-80 (direct mercury analyzer). This instrument directly analyzes a sample via thermal decomposition and subsequent conversion of mercury to its elemental state by means of catalytic reduction. The total mercury in the sample is then measured by a dual-cell atomic absorption spectrophotometer with total analysis time of approximately six minutes. Our results indicate that the DMA-80 is both accurate and precise method for determining mercury concentrations in fish tissue. In addition, the results appear to be more precise than traditional CVAAS techniques.

AUTOMATED SUPPORT OF TOC DATA QUALITY REQUIREMENTS

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Total organic carbon (TOC) analysis continues to grow as the analytical method of choice for pharmaceutical applications, including clean-in-place validation, Water for injection, purified water, and other critical purposes. The significant nature of these applications requires data validation using validation samples, spiked samples, and other techniques. System validation has become as important as the analyses themselves. These additional requirements place a heavy burden on the analyst and organization to monitor the devices for compliance.

This paper details system automation designed for validation monitoring of TOC measurements. Features include the ability to monitor calibration performance, verify compliance with user specified parameters, evaluate validation samples for accurate recovery, perform automatic system suitability tests, assess quality-control standards, perform verification through an IQ/OQ/PQ, and so forth. Additional elements include the capacity to meet compliance requirements for 21 CFR Part 11, as well as other regulatory guidelines. Automation of these steps frees the analyst to perform other duties while the instrument process samples, and protects the data and samples from instrument malfunction through its automated capability to verify user-defined pass/fail criteria.

SAMPLE SCREENING USING LC/MS AND LC/MS/MS LIBRARIES

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An LC/MS method will be presented for the screening of over 120 deleterious organic compounds in drinking water supplies. MS/CID and MS/MS libraries were created using a tandem mass spectrometer and MassLynx[®] software and subsequently converted to NIST format using the ChromaLynx[®] application manager. Samples were analyzed using an LC/MS full scan method with data collected at multiple cone voltages. The ChromaLynx application manager was used to automatically deconvolute chromatograms to identify sample components and search against the MS/CID library. The use of ChromaLynx eliminates the time-consuming task of manually processing the chromatographic and spectral data, searching the library and creating a list of tentative analytes. In addition, ChromaLynx has the ability to locate components in the sample which could be missed in a manual review due to close or coeluting components and to filter using retention time, ionization polarity, and cone voltage information.

Additional identification of sample components was carried out by LC/MS/MS. This analysis consisted of a product ion scan function for each of the components on the match list produced from the LC/MS analysis. ChromaLynx was again used to identify individual components and perform a search of the MS/MS library. Final confirmation of sample components was carried out by LC/MS/MS MRM analysis with TargetLynx[®] processing.

ONLINE SEPARATION AND IDENTIFICATION OF INORGANIC AND ORGANIC ARSENIC SPECIES FROM KELP EXTRACTS THROUGH LIQUID CHROMATOGRAPHY PARTICLE BEAM ELECTRON IONIZATION MASS SPECTROMETRY (LC-PB/EIMS)

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The widespread acceptance and increased use of nutritional supplements can be seen as the sale of natural supplements worldwide increased from \$8 billion in 1995 to \$19 billion in 2000 [1]. The speciation of metals in dietary supplements can be used as a fingerprint to the source of contamination of toxic heavy metals such as arsenic. The species-dependent toxicity of arsenicals requires analytical techniques capable of distinguishing the toxic (inorganic arsenic) from the non-toxic (organic arsenic) chemical forms [2]. The main limitations with atomic spectroscopy methods, however, are that they are only compatible to aqueous solvents and lack structural information about the detected compounds. At this point, one would desire an analytical technique that could provide structural, fragmentation, and elemental information (termed as comprehensive speciation) by using a single mass spectrometry ion source.

A simple isocratic high-performance liquid chromatography (HPLC) method was developed to separate inorganic and organic arsenic compounds (As(III) chloride, arsenobetaine (AB), and dimethyl arsenic acid (DMA)) on a C18 column with a mobile phase composition of water: methanol

(96 : 4). The eluted species were detected using a particle beam-electron ionization mass spectrometry (PB-EIMS) that can provide species-specific information. The flow was optimized at 0.7 mL min⁻¹. The addition of 0.1% (v/v) TFA improved the resolution of the mixture and the separation was accomplished in less than 7 minutes. Detection limits for As(III), DMA, and AB were found to be 0.13, 0.16, and 0.3 ng absolute in the SIM mode and 0.27, 0.41, and 0.11 ng absolute in the TIC mode, respectively. Subsequently, this method was applied to ethanolic kelp extracts, where the inorganic and organic arsenic species were separated and identified. These studies revealed that the majority of the arsenic (80–90%) is present in the tested kelp sample in the form of inorganic arsenic. Very minor amounts were present in the form of DMA (5–10% of the total As). There was no detectable level of AB. The total arsenic content in the ethanolic extract was determined by ICP-OES and validated by analyzing NIST SRM 3242 ephedra.

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RAPID ANALYSIS OF DRUGS AND METABOLITES DURING CLINICAL TRIALS USING SALIVA AND DISPOSABLE LAB-ON-A-CHIPS

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A typical drug requires 12 years of research and development to bring it to market. A critical step in the latter stages of development is human clinical trials to study pharmacokinetics and efficacy. This step represents a significant barrier in that there is a limited number of willing test patients. Patients must not only endure multiple withdrawals of blood, but also run the risk of adverse side effects, anemia, and possible HIV contamination. Saliva analysis has long been considered an attractive alternative to blood, but current methods involving extensive sample extraction followed by gas chromatography and mass spectrometry typically require 10–20 cc per analysis. In an effort to overcome this limitation, we have been developing lab-on-a-chip devices to both separate drugs and their metabolites from saliva and generate surface-enhanced Raman spectra (SERS). We have incorporated standard chromatography materials to aid separation, as well as a porous material containing metal particles that generate SERS. Generally, no more than a drop of sample is required and complete analysis can be performed in 5 minutes. The detailed molecular vibrational information allows chemical identification, while the increase in Raman scattering by six or more orders of magnitude allows detection of nanomolar concen-

trations. Measurements of basic drugs, drugs of abuse, and chemotherapy drugs will be presented.

THE MOBILE MONITORING SYSTEM (MMS): A USEFUL TOOL FOR ASSESSING AIR POLLUTION IN CITIES

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The first and essential step in controlling and mitigating air pollution is to quantify emissions of air pollution. The most important cause for air pollution in cities is urban traffic. In this situation, it is important to be able to monitor air pollutants, such as nitrogen oxides, sulphur dioxide, particulate matter, carbon monoxide, and volatile organic compounds, emitted by road traffic. Presently, in many Polish cities only fixed monitoring stations are installed, which measure the content of basic air pollutants in urban air. The measurement of air pollution alongside of communication lanes is very rare.

The paper presents the concept of a mobile monitoring system for main air pollutants in the cities. The novelty of the proposed system is connected with the fact that it can be used to monitor emissions from urban traffic along roads and those areas, where traditional monitoring stations cannot be placed.

In the proposed system, a mobile station may be attached to private cars, buses, trucks, and so forth. Stations in this system are small, portable structures and contain an electronic instrument used to measure, record, and transmit the concentration of various pollutants to the website. Weather conditions, such as temperature, and humidity are also measured. The proposed station should be cheap, reliable, requiring no frequent routine maintenance procedures like calibration, filter changes, and so forth.

The mobile monitoring system can determine representative air pollution in highly populated areas, the impact of communication sources of pollution, general background pollution levels directly affected by cars, and the highest pollution levels in the cities.

The proposed mobile monitoring system is a unique solution not only in Poland but also in the European Union.

NEW AUTOMATED SEPTUMLESS ON-COLUMN INJECTOR FOR CAPILLARY GC

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The cold on-column injector is widely considered, the most accurate and precise injection system, being able to ensure the preservation of sample integrity. Additionally, when manually operated, on-column injection has the important feature of being septumless and consequently free of

contamination due to septum bleed. This work presents the capabilities of a newly designed septumless on-column injector in combination with a robotic autosampler (TriPlus, Thermo Electron) that allows to extend this feature to an automatic system. The injector, along with special sample vial closures and a solenoid-valves-based syringe washing station, avoids any contact between sample and silicon septa, preventing any siloxane interfering peaks.

Performances of this system are illustrated through typical on-column applications such as triglycerides analysis and SimDist characterization of petroleum fractions. Analysis of real food samples such as edible oils and their triglycerides characterization is also presented. These applications are problematical when using hot injectors since sample discrimination, during sample transfer from the needle into the injector and from the injector into the column, may produce unreliable chromatograms and subsequent quantitations.

OPTIMIZATION OF THE EXTRACTION STEP OF BUTYLTIN COMPOUNDS BY SOLID PHASE MICROEXTRACTION: INFLUENCE OF PRESSURE AND DIMETHYL SULPHOXIDE CONCENTRATION

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A method based on headspace solid phase microextraction (HS-SPME with gas chromatography and pulsed flame photometric detection (GC-PFPD)) has been investigated for simultaneous determination of monobutyltin MBT, dibutyltin DBT, and tributyltin TBT. The effects of pressure and presence of dimethyl sulphoxide (DMSO) during HS-SPME sampling were evaluated using modified 50 mL conical flasks and magnetic stirring at room temperature. The coated phase used in this study was polydimethyl-siloxane (PDMS, 100 μm). The optimal pressure was 0.3 bar, obtaining increasings of 30%, 39% and 50% on the extraction yield of MBT, DBT, and TBT, respectively. The extraction and pre-concentration steps at this pressure were optimized by experimental design methodology. The screening step showed that three variables were significant: sample volume, agitation time, and DMSO concentration. The optimized values are 15 mL, 25 minutes, and 33% (v/v), respectively. Agitation time was lower than applied at normal pressure (40 minutes). A comparison of the extraction yield using fibers with different stationary phases (PDMS/DVB 65 μm , CAR/PDMS 75 μm , DVB/CAR/PDMS 50/30 μm) showed that the optimal fiber for MBT and DBT extractions was the PDMS fiber and for TBT was the DVB/CAR/PDMS fiber. The analytical performances determined by using standard solutions and tripropyltin as internal standard were linearity range LOQ-80 ng Sn and detection limits for MBT 0.1 ng Sn and for DBT and TBT 0.03 ng Sn. Sensitivities were three times higher than those obtained at 1 bar and 40 minutes of agitation. Repeatability RSD (%) ranged between 15% and 0.2% for solu-

tions of 2 to 75 ng Sn. This methodology was applied to the analysis of Chilean fish samples.

ACKNOWLEDGMENTS

The authors are grateful for the financial support of CONICYT, Project 1050417, and PUCV-DI, 125 774.

SIMULTANEOUS DERIVATIZATION/PRECONCENTRATION OF VOLATILE ALDEHYDES WITH FIBER-PACKED SAMPLE PREPARATION DEVICE DESIGNED FOR GAS CHROMATOGRAPHIC ANALYSIS

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A novel in-needle sample preparation device was developed in this work, which is designed for the GC analysis of aldehydes and ketones commonly found in typical in-house environments. Simultaneous derivatization with 2,4-dinitrophenylhydrazine (DNPH) was made during the sampling process of the gaseous sample, and the derivatized analytes were desorbed by passing a small amount of organic solvent through the extraction needle in the heated GC injection port. The reproducible preparation of the extraction needle was established along with the repeatable derivatization/extraction that ensures the successful determination of aldehydes. Basic extraction performance of the fiber-packed extraction needle has been evaluated along with the optimization of several experimental parameters on the derivatization, extraction, and desorption. Taking into account the applications including the in-house environment analysis, the storage power of the needle device for the extracted analytes under the room temperature was also studied. The results demonstrated the successful applications of the fiber-packed extraction device for the sample preparation of gaseous sample of aldehydes, and the future possibility of the extraction device for the analysis of in-house environments.

SOPRANE, POWERFUL MONITORING SOFTWARE FOR MICROGC

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Why an alternative to standard *MicroGC software*? Standard chromatographic software is not adapted to *MicroGC* specificity. *MicroGC* is more and more used for monitoring application and the software has to provide specific features in accordance with *MicroGC* technique.

A complete solution means powerful software from the analysis programming to the result report. *Soprane* controls all of the following analysis steps:

- (1) sampling: 1 to 16 *stream selections*, with multiposition valves, solenoid valves, and ejectors, personalize and create your own sampling sequence;
- (2) automation: automatic *multilevel* calibration, alarms to personalize your own sampling sequence, acquisition sequence, bake-out scheduling (you can create and edit methods for integration and identification, and run single analyses, sequences, and calibrations; the result report is easy to personalize with normalization, totals, unknowns, groups, etc.);
- (3) *specific calculation* (ICV, SCV, density, M, Wobbe index, viscosity, etc.) are available as personalized calculations edited directly in Excel (possibility of VBA macro use);
- (4) management of *defects* and *alarms* (with relay board);
- (5) visualization and results archiving (values and *trends graphs*);
- (6) report printing (manually or automatically);
- (7) *data transmission* (4–20 mA and serial link) for alarms and values is available.
- (8) fieldbus interface for data transmission (*Modbus, PROFIBUS* etc.);
- (9) *DDE connectivity* for automated applications (use your own software and drive Soprane);
- (10) prerun and postrun for personalized applications;
- (11) *external data reading* (pressure, temperature, etc.), and integration in specific calculations;

Chromatograms comparison and mathematical operations are possible on the signal.

CHARACTERIZATION OF FLAVORS AND FRAGRANCES IN BEVERAGES BY SPME-COMPREHENSIVE 2DGC

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The analysis of Flavors and Fragrances in complex matrices is one of the ideal applications where it is possible to take advantage of the unprecedented separation power delivered by comprehensive 2DGC. Several papers presented over the last decade have explored the capability of this innovative technique to provide target components analysis as well as detailed characterization of flavors in various types of beverages.

This work relates to the coupling of comprehensive 2DGC with SPME for this type of analysis, fully automated by the TriPlus autosampler (Thermo Electron) on the TRACE GCxGC equipment (Thermo Electron), managed through a dedicated data system (Hyperchrom, Thermo Electron).

Several examples of analysis of various beverages, both alcoholic and nonalcoholic, allow to appreciate the advantages of the enormous chromatographic separation together with the simplicity of the solventless sample preparation technique applied.

On top, the mechanism of agitation, specifically developed on the TriPlus autosampler in order to reduce the mechanical stress on the SPME fiber, allows to significantly extend the average lifetime of standard fused silica fibers. The presented data show that long sequences of samples can be run using the same fiber, further expanding the productivity of the whole analytical cycle.

THE ANALYSIS OF CHLORINATED HYDROCARBONS IN AIR AT PPB LEVELS UTILIZING AN AUTOMATED SAMPLE ENRICHMENT AND DESORPTION DEVICE COUPLED WITH A MEMS-BASED GAS CHROMATOGRAPH AND TUNABLE DIFFERENTIAL MOBILITY DETECTOR

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The toxic effect of chlorinated hydrocarbons has been well known and characterized for many years. In order to determine whether airborne concentrations of chlorinated hydrocarbons will have an adverse effect, precise measurements have to be taken at many remote points to get a representative picture. This is particularly true in urban and large industrial environments.

Devices have been developed to measure chlorinated hydrocarbons at low PPB levels but none has been really optimized for remote monitoring. Those devices have been typically designed for use in the laboratory and present some formidable limitations when used in remote locations. Some of the key limitations include lack of long-term stability (PID), high maintenance costs (GC/MS and to some extent GC/FID), or large physical size and weight. Some IR-based devices are used in remote locations but, unfortunately, require a long measurement path length and cannot be used during inclement weather.

To address these limitations, preconcentrator/sample enrichment device, MEM-based chromatograph (Micro GC PRO), differential mobility detector (DMD), and high-resolution capillary column were combined in a system optimized for the measurement of chlorinated hydrocarbons in remote nonlaboratory environments. The system can selectively measure components at low PPB levels. The on-board signal processing, peak integration, and data processing capability of the Micro GC PRO provide highly accurate and repeatable BTX measurements in remote locations without the need for skilled operators.

Details from a study commissioned to validate the system for remote measurements of chlorinated hydrocarbons at low ppb levels will be presented and discussed.

OPTIMIZATION OF GAS CHROMATOGRAPHIC DETERMINATION OF TRANS FAT

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The declaration of trans fat content is now required on food labels in the US. Trans fat content is expressed on a per serving basis and is calculated by adding the content of all fatty acids having at least one nonconjugated trans double bond. Fatty acids analysis is generally achieved by gas chromatography using long (100 m) polar capillary columns such as Supelco SP 2560, Varian CP-Sil 88, or Agilent HP-88. In order to properly report trans fat content, analysts are required to enhance the existing methods of analysis by optimizing fatty acid separation, increasing the number of fatty acids analyzed, and investigating analytes present at low concentrations. None of the official methods of analysis for routine quantitation of fatty acids in foods currently includes any information regarding limits of detection and quantitation. In the current work, we are addressing critical aspects of trans fatty acids quantitation in order to obtain more accurate values for total trans fat content. Comparisons among separations achieved using different GC columns and lower quantifiable values for trans fatty acids will be discussed.

OPTIMIZING HPLC PARTICLES AND COLUMNS FOR SEPARATION SPEED

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Much recent emphasis has been placed on making major reductions in particle size to significantly improve HPLC performance at the expense of pressure; however, such extreme measures are seldom practical for the industry to absorb quickly. In the meantime, workhorse HPLC columns for the industry are still made predominantly with 5 μm and 3 μm particles. Too little has been done to evaluate the gains that can be achieved by the important step of moving applications from 5 μm to 3 μm particles. The design and performance of 3 μm particles will be compared to other smaller particles in short column formats. While silica is the most common and useful substrate today, the benefits of other substrate platforms will also be reviewed.

Choosing a substrate with the right particle size is not the only important step in optimizing separation speed. Pore size, pore structure, stationary phase chemistry, and column hardware all play an important role in determining separation performance, sample throughput, and column lifetime. Column performance will be compared under both isocratic and gradient conditions in order to judge the true value of various particle sizes and bonded phases under practical use conditions. High-speed LC-UV and LC-MS applications will be shown for various columns and particle sizes using both conventional LC systems having 400 bar pressure limits and new LC systems having 1000 bar pressure limits. The practical limits of what separation speeds can be achieved today with conventional LC systems and columns prepared with 3 μm particles will be clearly demonstrated. The potential limits of what speeds might be achievable

with new LC systems and smaller particle technology will be explored.

HIGH-THROUGHPUT ANALYSIS OF THE CHEMICAL COMPOSITION DISTRIBUTION OF POLYOLEFINS BY CRYSTALLIZATION ELUTION FRACTIONATION

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The new technique crystallization elution fractionation, which combines TREF and CRYSTAF, results in a very fast analysis time that can be used successfully in the high-throughput analysis of the chemical composition distribution. A new compact and robust equipment has been built to analyze 50 samples/day in a fully automated mode with very low solvent consumption. The easy incorporation of a viscometer and/or light scattering detectors provides additionally molar mass composition interdependence information.

FAST SCREENING OF SECOND VIRIAL COEFFICIENT FOR PROTEIN FORMULATION WITH LIGHT SCATTERING DETECTORS

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Aggregate formation is an important concern in the purification, formulation, and storage of protein therapeutics. The integrity of protein preparations is often monitored by HPLC using UV and RI detection. However, the characterization of aggregates present at very low levels poses a significant challenge to current HPLC technology. For many cases, the protein samples are separated by HPLC to determine the trace of various components such as monomer, dimer, and aggregates (multimers). It is a very time-costly process and almost impossible to predict without running HPLC. Therefore, there is a need to develop a model or method to screen the formulations in a time-efficient way. The virial coefficient is often used to determine the interactions between protein molecules and their interaction with the solvent molecules, which is usually performed by static light scattering (SLS) detectors and tells about the repulsive or attractive interactions between protein molecules and solvent molecules. Light scattering can be used as a highly informative tool to determine the second virial coefficient for an extensive determination of the different types of aggregates. This is helpful in a faster selection of a favorable formulation and therefore can be used as a fast screening technique without the need of conducting an accelerated stability study for the purpose of formulation development.

FULLY AUTOMATED LOW-LEVEL METHODS FOR THE ANALYSIS OF TOTAL PHOSPHATE, TOTAL CYANIDE, DISTILLED PHENOL, AND ANIONIC SURFACTANTS BY FLOW INJECTION ANALYSIS

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Flow injection methods for low-level determination of total phosphate, total cyanide, and distilled phenol have been developed. All three methods can be quantitated to 2 ppb. These methods are all fully automated and require no sample preparation. We have also developed an anionic surfactants method that can quantitate to 10 ppb sodium dodecyl sulfate (SDS) and 25 ppb linear alkyl sulfonate (LAS) without sample preparation or extraction. Because these are high-throughput methods (>12 samples per hour) and no sample preparation is required, these methods can save hours of time a day when compared to manual methods.

DESIGNING AND IMPLEMENTING A HIGH-THROUGHPUT, DECISION-BASED SOFTWARE SYSTEM FOR AUTOMATED PREPREPARATIVE HPLC/MS ANALYSIS, METHOD DEVELOPMENT, AND PREPARATIVE SEPARATION

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Developing HPLC methods for complex mixtures of chemicals or pharmaceuticals is a time-consuming and knowledge-intensive task. This task can be especially challenging when dealing with high-throughput analysis required from synthesis of libraries of compounds, where instead of designing an HPLC method for a single sample, HPLC methods are needed for single and multiple plates, each containing up to 96 samples. Sometimes, there are similar structures between the samples on a plate, and at other times each sample contains unique compounds from different synthetic chemistry laboratories.

When physical property data are available for the compounds such as various pKa data, logD values at different pHs, solubility information, structural information, and molecular stability, the samples can be classified to determine a priori which HPLC methods should provide reasonable separation. The expert knowledge used to perform this classification involved, creating detailed paper-based or Microsoft Visio-based decision trees, which were then automated with software. We refer to this as automated "pre-preparative analysis."

The next task involves running and analyzing the results of the predicted HPLC methods for each sample on the actual HPLC equipment. In some cases, the results can be easily scaled to subsequent preparative separation and in other cases, the results indicate that further HPLC method

development is required. Again, the expert decision making is taken from static diagrams to real-time, online software automation. This presentation will show practical examples of how software decision trees were incorporated into a walk-up/open access system for multisample, pre-preparative analysis, rapid method development, and preparative separation.

SIMULTANEOUS DETERMINATION OF DISSOLUTION RATE, ASSAY, CONTENT UNIFORMITY, IDENTIFICATION, AND RELATED COMPOUNDS USING AN HPLC SYSTEM

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Stability and formulation testing can be a lengthy and labor intensive process if each test is performed individually. These tests normally involve multiple sample preparation, extraction solvent preparation, and dissolution media preparation. An alternative approach is to utilize the dissolution test to acquire all data required for stability and formulation testing. This poster will present the methods used and data acquired including implementation and validation of simultaneous determination of dissolution rate, assay, content uniformity, identification, and related compounds using a Waters 2695D HPLC system.

A MINIATURIZED ONLINE SPE-LC SOLUTION FOR DIRECT ANALYSIS OF DRUGS IN SMALL VOLUME PLASMA SAMPLES

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In recent years, online SPE-LC has gained popularity as an alternative to manual pretreatment of complex samples such as blood, plasma, and urine. A dedicated system for this approach comprises two gradient pumps in one enclosure, a column switching valve, and a powerful software solution for easy setup and operation.

A dedicated SPE-column as an integral part of the total analysis system is mandatory for a bioanalytical online SPE-LC system. It has to repeatedly deplete the matrix without unselective accumulation of matrix components and allow target analyte(s) extraction with unchanged selectivity/capacity despite multiple uses. The so-called restricted access materials (RAMs) fulfil these requirements [1].

Online SPE-LC with 4 mm ID SPE-column used in connection with 4–4.6 mm ID HPLC columns requires injection volumes of up to 500 μ L in order to achieve appropriate limits of quantitation. Biofluid samples are limited in volume and must often serve for several diagnostic determinations. In addition, the flow rates on these column dimensions are not ideal for electrospray MS.

We present a novel, miniaturized (20×1 mm ID RAM SPE-column and 50×2.1 ID mm analytical column), online SPE-LC solution for rugged and reliable analysis of drugs in biofluids. This reduces cycle time 2-fold, solvent consumption 8-fold, and injection volume 5-fold compared to common online SPE-LC. Overall, the sensitivity is enhanced approximately 8-fold.

As an application, we show the fully automated analysis of antimycotic drugs in human plasma.

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COMPUTER-AIDED IMPLEMENTATION OF INSTRUMENT METHODS FOR ONLINE SPE-LC

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Online SPE-LC is used for convenient, fully automated sample preparation of complex samples combined with inline separation and detection of the target analytes. During online SPE-LC method development, several partial procedures like matrix elution time (t_M), analyte breakthrough time (t_A), and analyte transfer time (t_T) have to be coordinated. Based on these parameters, the correct valve switching times must be defined. In addition, two gradient control programs need to be created. These requirements represent a barrier for users and therefore need to be overcome to allow for a broader adoption of this powerful technique.

This poster shows how this barrier can be removed by an intelligent software solution. The software approach uses a wizard which calculates from method-dependent user input (e.g., t_M , t_A , and t_T) appropriate column switching times, and then generates a suitable instrument method accordingly.

The computer-aided implementation of online SPE-LC methods greatly simplifies sample preparation by

- (i) accelerating the process of creating online SPE-LC methods;
- (ii) improving reproducibility of the recovery;
- (iii) reducing user errors;
- (iv) allowing automation of sample preparation without special user training;
- (v) Increasing laboratory productivity.

NOVEL AUTOMATED PRESSURIZED MICROWAVE-ASSISTED SOLVENT EXTRACTION

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Microwave-assisted solvent extraction techniques have become accepted as a rapid and low solvent use "green" tech-

nology. Pressurizing the extraction solvent allows temperatures to be achieved in excess of the atmospheric boiling point and thus increases the rate of partition of the analyte into the liquid phase. Of course, care must be taken to ensure that the temperature is not sufficient to degrade the analyte of interest.

This paper will introduce a novel approach to automation in microwave-assisted solvent extraction. A semiopen focused microwave system will be shown to offer significant advantages in terms of extraction time, ease of use, and flexibility.

Analytical data will be presented for solvent extractions of polymers and plastics for environmentally important additives. Enhanced flexibility and ease of use will be demonstrated along with low solvent usage. This novel, sequential sample processing instrument can rapidly extract up to 96 samples with each sample under full temperature and pressure control. A comparison will be made of extraction efficiency for samples that are stirred and unstirred and a range of sample sizes to extract solvent volume ratios studied. Results will be presented which show a significant saving in time and solvent volume for extractions of this type.

ONLINE AND OFFLINE APPLICATION OF MICRO-SPE

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Solid phase extraction (SPE) has revolutionized sample preparation methodology for diverse sample types. In many cases, variations on the technique offer enhanced recovery, greater opportunity for speciation, and a reduction in solvent and sample consumption. Unlike solvent extraction, SPE is generally easy to automate for offline use. The simple adaptation of SPE for online use has not been as successful as its uptake for offline applications.

Recognizing that the primary purpose of most SPE methods is solvent exchange (e.g., the extraction of an aqueous sample for GC inlet) or some form of matrix cleanup (e.g., desalting or removal of endogenous materials), the further development of SPE for online use becomes a directed exercise. Miniaturization of SPE into the sample injection system allows for a much smaller portion of the sample to be extracted and for the whole of the extract to be injected onto the chromatographic column for separation. In many cases, such an approach allows the same level of sample concentration to be achieved as possible with offline conventional SPE. Providing the miniaturization does not approach the same scale as the pseudoplates of the sorbent; micro-SPE may be readily adapted from established SPE methods.

Using simple applications, we demonstrate the usefulness of micro-SPE for different combinations of sample type and sorbent chemistry prior to analysis by GC or LC techniques.

The micro-SPE apparatus is suitable for online use in GC, HPLC, and for sample preparation prior to other analytical techniques including immunoassay and offline nondestructive spectroscopic analysis by NMR, IR, and other methods.

AUTOMATED SEQUENTIAL PRESSURIZED DIGESTION USING A NOVEL FOCUSED SEMI-OPEN MICROWAVE INSTRUMENT

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Pressurized microwave digestion technology has become widely accepted as a rapid and convenient method of sample preparation for metals. However, the nature of microwave interaction with the acid/sample matrix forces analysts with widely varying samples to run small batches of similar samples. Recent advances in high-throughput technology have increased batch size but they have not addressed the fundamental issue of control of this batch process.

This paper outlines a new sequential approach using a novel semi-open focused technology to this problem for analytes with samples of widely varying type requiring widely varying methodology.

Novel focused, semiopen, microwave instrumentation is presented with an inbuilt control network allowing sequential processing of multiple samples requiring multiple reagents and methodologies. An automated control system allows a sample weight to vessel volume ratio far in excess of any previous technology, reducing reagent use and maximizing analyte concentration. Results will be presented demonstrating the use of the equipment for total digestion or dissolution of reference materials, at elevated pressures and temperatures for multiple samples automatically, in sequence under multiple sets of reagents and conditions. Additionally, one organic reference material (TORT-2) will be presented, digested with the same methodology, over an extremely wide sample size range illustrating the improvement in sample-to-volume ratio accomplished over traditional microwave batch systems.

APPLICATION OF A NOVEL AUTOMATED SPE PROTOCOL USING A MULTISORBENT METHOD DEVELOPMENT PLATE FOR NEUTRAL, ACIDIC, AND BASIC DRUGS

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Rapid method development is the most sought after segment of sample preparation during the analysis of pharmaceuticals from biological matrices, especially in performing analysis of ADME (absorption, distribution, metabolism, and excretion) samples during new drug development. Solid phase extraction (SPE) is the preferred technique for processing high volumes of samples due to its amenability for automation and its ability to concentrate subnanogram level analytes while simultaneously delivering clean extracts. The major obstacle to the use of SPE for this type of high-throughput analysis is determining the most suitable sorbent and extraction conditions for a particular analyte. Screening sorbents

individually for optimal recovery and cleanliness are time-consuming and tedious.

In this communication, we present a simplified, nevertheless exhaustive approach to optimizing the sorbent and conditions for the SPE of analytes from biological matrices. Using a MULTIPROBE II automated liquid handling system and a recently released 96-well polymeric method development plate, we developed a universal screening protocol for defining the sorbent and conditions for SPE in less than one hour. The 96-well plate contained four polymeric sorbents of divergent surface chemistry, which cover hydrophobic, polar and ion exchange interactions between them. Using carbamazepine as a neutral probe, atenolol, metoprolol, and promethazine as representatives of polar and hydrophobic basic probes, and indomethacin as an example of acidic probes, we demonstrate that the universal protocol can cater to all kinds of analytes in furnishing optimal conditions for the solid phase extraction from any biological matrix.

OMEGA 3: QUALITY-CONTROL, ODOR MASKING, AND AGING MONITORING WITH AN ELECTRONIC NOSE

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Ingredients with high concentration of omega 3 are increasingly used in the food and beverage industry. However, this ingredient can bring an undesirable odor/aroma to the final product.

Consequently, it is considered to be of upmost importance for both omega-3 manufacturers and users to monitor the organoleptic features of this additive.

Usually, food ingredients are evaluated by human sensory panels, which can be unpleasant and time-consuming.

An electronic nose based on gas sensors arrays, the FOX instrument, has been used to assess Omega 3 sensory quality and to perform aging studies.

Analyzed omega-3 powder addressed to infants included samples at 2 different times: $t = 0$ (freshly produced) and $t = 2$ months under various storage conditions (air or nitrogen).

Those samples were assessed by sensory panel (scoring scale between 1 and 2) and analyzed with the FOX electronic nose.

It results in a clear discrimination between samples used as control Omega 3 powder and samples to be analyzed and a clear differentiation of samples according to storage conditions.

After building a calibration curve with the FOX electronic nose, the model showed a good correlation with sensory panel scores (correlation coefficient > 99%) and allowed to predict unknown powders scores.

The FOX electronic nose can bring about the ability to screen a large number of samples to check their quality. Thus companies can reduce time and costs of analysis chain process, and significantly improve the product-quality consistency.

IN VITRO CONTINUOUS AMPEROMETRIC MONITORING OF SEROTONIN RELEASE FROM ENTEROCHROMAFFIN CELLS OF THE GUINEA PIG ILEUM

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Enterochromaffin (EC) cells play a key role within the enteric nervous system. EC cells function as sensory transducers that respond to mechanical or chemical stimulation of the mucosa by releasing serotonin. Serotonin then acts on mucosal endings of enteric primary afferent neurons and extrinsic primary afferent neurons to initiate motor reflexes and intestinal sensation. Development of stable and reliable techniques for measurement of serotonin would be of great value in understanding the normal physiology of EC cells and their contribution to control of gastrointestinal function and visceral perception.

Serotonin is easily oxidized and thus can be detected using electrochemical methods. However, a major complication with stable detection of serotonin *in vitro* or *in vivo* is the rapid electrode fouling that occurs caused by adsorbed reaction products. To overcome this problem, we have used boron-doped diamond (BDD) microelectrodes which help to overcome fouling effects compared to conventionally used carbon fiber microelectrodes.

We have used amperometry detection at +700 mV versus Ag—AgCl for the *in vitro* detection of serotonin from EC cells, where we show that BDD microelectrode has much better suited such measurements compared to carbon fiber microelectrodes. We will show how various different stimulus (chemical, electrical, and mechanical) affect the amount of serotonin release and its possible physiological meaning. We will also show a study of how responses are varied from neonatal to adult animals, which will have implications on studying how stages of development could alter gut mobility.

METABOLIC PROFILING IN DIABETES USING ADVANCED 1H NMR-BASED MULTIVARIATE STATISTICAL ANALYSIS

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Diabetes has been a major health concern due to the constantly increasing number of affected people worldwide. Diabetes accounts for nearly 17 million patients in the United States alone. Diabetes, if untreated, will cause slow deterioration of heart, kidneys, eyes, feet and skin, nerves, teeth, and gums. Blood glucose has been the most reliable indicator of the onset of diabetes. However, it is of interest to develop earlier indicators of its onset. Metabolic profiling in diabetes promises immense potential for better understanding the biological processes associated with diabetes that might make significant impact on its control and therapy. We per-

formed 1 H NMR-based multivariate analysis of serum from prediagnosed diabetes patients and healthy controls. 1 H NMR experiments on diabetes and control serum samples were performed on a BrukerBiospin Avance 500 spectrometer using water presaturation by suppressing macromolecular signals using Carr-Purcell-Meoboom-Gill pulse train. Two-dimensional J resolved spectra were also obtained separately for each sample. The real part of the processed data and 450-tilted F2 projection of J-resolved spectra were subjected to multivariate analysis using several analysis methods after removing the regions containing water (4.8 ppm) and reference (0 ppm) signals. Excellent separation of diseases and control samples into clusters was observed in the analysis and the separation showed better improvement when the data were subjected to principle component analysis (PCA) using mean centered variables filtered using the thresholds determined from correlation. A series of complementary markers was identified. Correlation of these markers with glucose will also be discussed.

MONITORING DIET EFFECTS FROM BIOFLUIDS AND THEIR IMPLICATIONS FOR METABOLOMICS STUDIES

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The effect of diet on metabolites found in rat urine samples has been investigated using nuclear magnetic resonance (NMR) and a new ambient ionization mass spectrometry experiment, extractive electrospray ionization mass spectrometry (EESI-MS). Changes in diet at 24-hour intervals led to perceptible changes in the spectral data of both NMR and MS. Urine samples from rats with three different dietary regimens were readily distinguished using principal component analysis (PCA) on metabolites detected by NMR and MS. To observe the effect of diet on metabolic pathways, metabolites related to specific pathways were also investigated using multivariate statistical analysis. Discrimination is also observed by making observations on restricted compound sets. Principal component analysis (PCA) was used to separate the rats into groups according to their different dietary regimens using the full NMR, EESI-MS data, or restricted sets of peaks in the mass spectra corresponding only to metabolites found in the urea cycle and metabolism of amino groups (UCMAG). By contrast, multivariate analysis of variance (MANOVA) from the score plots showed that metabolites of purine metabolism obscure the classification relative to the full metabolite set. These results suggest that it may be possible to reduce the number of statistical variables used by monitoring the biochemical variability of particular pathways. It should also be possible by this procedure to reduce the effect of diet in the biofluid samples for such purposes as disease detection.

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ONLINE DETERMINATION OF ARSENIC USING A QUARTZ CRYSTAL MICROBALANCE

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Arsenic contamination of surface and ground water is an ongoing problem that places the health of people around the world at risk. Found in abundance in the earth's crust, As mobilizes over time and leaches into surrounding bodies of water. Consequently, there is a need for methods to reliably monitor arsenic in drinking water. While several laboratory-based methods exist for the determination of arsenic; those methods are costly, time-consuming, and require trained operators. Field test kits have been developed as a relatively simple, low-cost method for the determination of arsenic, however, these methods tend to produce unreliable results at concentrations near the maximum permissible limit of 10 mg/L set by the WHO. The method discussed in this work involves the determination of As(III) and As(V) in water. A portable sensor based on a quartz crystal microbalance (QCM) will be employed. QCM sensors offer the advantage of being lightweight and relatively simple to operate; however, they are inherently nonselective. The selectivity of the surface will be enhanced using a functionalized polymer to specifically bind As(V). A flow injection system will be used in conjunction with a flow cell apparatus for online determinations. The sensor will be evaluated for its ability to determine arsenic in solution and in the gas phase. The selectivity of the sensor, along with analytical figures of merit, will be determined. Once the appropriate parameters are optimized, the performance of the sensor will be determined using standard reference materials and real-life water samples collected from local bodies of water.

DEVELOPMENT OF LASER ABLATION-INDUCTIVELY COUPLED PLASMA-ATOMIC EMISSION SPECTROMETRY (LA-ICP-AES) TECHNIQUE FOR MONITORING VITRIFICATION OF HANFORD RADIOACTIVE WASTE

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Hanford tank waste treatment and immobilization plant (WTP) initiated the development of laser ablation inductively coupled plasma atomic emission spectrometry (LA-ICP-AES) for elemental analysis of sludge samples during

vitrification of high-level radioactive waste (HLW). This alternate technology will support rapid turn-around time (TAT) requirement for process control analysis. Simulant sludge samples were converted to glass pucks and analyzed at Battelle Pacific Northwest Division (PNWD) and Savannah River National Laboratory (SRNL) using different UV laser units and ICP-AES instruments. The studies at PNWD used New Wave UP-266 laser and Thermo Jarrel Ash (TJA) axial ICP-AES while SRNL utilized Cetac LSX-200 laser in conjunction with Perkin-Elmer Optima 3000 radial ICP-AES. The internal standards used were also different in two studies. PNWD utilized Cu and SRNL used Sc and Si for internal correction. Irrespective of different instruments and methods, similar results were obtained from both laboratories. The precision of analysis was <10% RSD for majority of elements of interest. The accuracy for elements at concentrations greater than 3xMDL (method detection limit) was within +10% of true value. Both laboratories demonstrated the feasibility of TAT of <9 hours for LA-ICP-AES analysis and showed data comparability to conventional dissolution ICP-AES method. The application of the developed method for remotely ablating HLW samples in a hot cell environment will be discussed.

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SIMULTANEOUS DETECTION OF MULTIPLE BACTERIAL CELLS USING FLUORESCENT NANOPARTICLES

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The rapid and sensitive determination of pathogenic bacteria is important for medical diagnosis, food safety, and today's fight against bioterrorism. In this work, we have employed the emerging nanotechnology to develop a highly specific, rapid, and sensitive method for simultaneous identification and quantitative determination of target bacteria, primarily, *Pseudomonas aeruginosa* (PA) and *Klebsiella pneumoniae* (KP). The principle of the method is to use fluorescent nanoparticles as labeling reagents to emit signals at different wavelengths for different bacterial cells. The nanoparticles were developed based on fluorescence resonance energy transfer (FRET). Therefore, when a single excitation wavelength is used, multiple emission wavelengths will appear to indicate different target bacteria. The principle discovered in this study could be extended to the rapid and sensitive determination of various bacteria and viruses.

The nanoparticles first conjugated with specific antibodies to form conjugates of nanoparticle-antibody (NP-Ab). Then, the NP-Ab conjugates were used as target bacteria identifiers. Due to the strong fluorescence of the nanoparticles, the identifiers provide high fluorescence signals for trace amount of target bacterial cells. Preliminary results

have demonstrated that the antibody conjugated nanoparticles can readily and specifically identify target cells down to a single one within 30 minutes.

REAL-TIME MONITORING OF ORGANIC REACTIONS AND PHARMACEUTICAL PROCESSES VIA HPLC

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Valuable information can be obtained by real-time monitoring pharmaceutical processes such as organic reactions and dissolution testing. Example information includes reaction progress, reaction mechanisms, impurity formation, reaction optimization, and dissolution rates. Despite the need, there are presently few attractive options for monitoring pharmaceutical processes. FTIR probes have been available for some time, but the technique's limited specificity severely limits its application to a small fraction of processes. HPLC is a much more universal technique than FTIR; however this requires very time-consuming human intervention for sample withdrawal and dilution. This manual process is also prone to human error.

We have developed a system that automates the sampling and dilution processes and allows for unattended monitoring of pharmaceutical processes by capillary HPLC. The system includes an inline autosampler, capillary HPLC system, and peak-analysis software. The system is contained on a compact cart for portability. Real-world processes can be monitored with sample volumes of tens of microliters and chromatographic results as frequent as 2 minutes or less.

The authors will describe real-time monitoring of reaction progress from organic reactors using an inline autosampler and dilution device coupled to a capillary HPLC. Analytical figures of merit for the system will be presented.

A NOVEL, PROGRAMMABLE, AND UNTETHERED SYRINGE DEVICE CREATES AN INNOVATIVE SAMPLE PROCESSING PARADIGM FOR LABORATORY AUTOSAMPLERS

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New drugs can take twelve years and \$850 MM to develop partly due to transferring underqualified candidates into development. To vet candidates earlier, ADME-Tox and other studies are moved forward into discovery which increases sample loads 10X from 600 to 2000 samples/day compared to development. Modern LCMS systems keep pace by providing up to five-order dynamic range and run-to-run capabilities approaching 1200 samples/day making autosamplers the throughput and integrity bottleneck.

This paper describes a novel autosampler based on newly invented sample handling and injection technology that effectively decouples carryover from throughput. The invention utilizes autonomous, untethered, asynchronously operating syringes which have 8 mm outer diameter, weighs 26 grams, requires no electrical or fluidic tethers, can deliver against hundreds of psi, possesses radio frequency identification (RFID) technology for volume, calibration, and usage data, and contains on-board memory to store and record sample workflows making workflow management fully automated and sample-centric. These features enable a new autosampler workflow model with substantially greater throughput via parallel operation of multiple syringes, real-time workflow, chain of custody, audit trail monitoring, reduced processing errors, and built-in fault tolerance and recovery.

While this new approach can be broadly applied to any autosampling application, we demonstrate its use in ADME-Tox studies providing 1800 samples/day throughput with 0.005% carryover (reserpine) compared to 250 samples/day with >0.05% carryover (reserpine less than four-order dynamic range) as tested on 3 conventional autosamplers. Two independent pharmaceutical case studies demonstrate throughput and carryover levels remain consistent throughout a five-day evaluation period.

OPTIMIZING THE MORPHOLOGY OF NANOSTRUCTURED SERS SUBSTRATES CREATED VIA ELECTRON BEAM LITHOGRAPHY AND PHYSICAL VAPOR DEPOSITION

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Signal enhancement observed in SERS is attributable to the presence of noble metal nanostructures on substrate surfaces. The rational development of SERS active substrates depends critically on the homogeneity and intensity of surface plasmon resonances, properties that are strongly dependent on both the morphology and dielectric properties of the metals and composite materials making up the SERS substrates. Enhancement can be controlled by the shape, size, and spacing of metallic nanoparticles. Previous studies in our group have shown that elliptical arrays have promising geometries for this purpose. Using electron beam lithography, we fabricate close-packed arrays of ellipses with aspect ratios ranging 50:100–300:300 nm. The arrays are composed of a negative photoresist that, once the lithography process is complete, is coated with a noble metal through physical vapor deposition. In this work, studies are done to determine the optimum thickness and deposition rate of noble metal for these substrates. It is then found that by altering the aspect ratio of ellipses, and by changing the gap between ellipses in an array, SERS enhancement can be tuned. The underlying nanostructuring of the SERS substrates greatly improves the collected SERS signal as can be seen when data are collected “on-pattern” versus “off-pattern.” The Stokes responses of

these lithographically produced nanopatterns are collected with SERS, while scanning electron microscopy is used to examine pattern surfaces after lithographic development and after noble metal deposition.

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