Non-destructive forensic document examination of ballpoint cationic inks by blotting-capillary electrophoresis

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We present a nondestructive chemical analysis method for examining documents written with black ballpoint pens. A simple and convenient ink-blotting technique was developed to extract trace amounts of ink from original document surfaces onto a nylon membrane filter (NMF) using isopropyl alcohol, effectively avoiding smudging, blurring, or physical damage. Small circular sections of the blotted NMF were punched, extracted with methanol, and analyzed by nonaqueous capillary electrophoresis (NACE) using a methanol-based run buffer optimized for water-insoluble cationic dyes commonly found in ballpoint inks. To enhance detection sensitivity, large-volume sample stacking with an electroosmotic flow pump (LVSEP) was employed as an online preconcentration technique. LVSEP was performed under normal polarity using a polybrene-coated capillary. A one-time rinsing with water for 6 h following the coating process was essential for achieving stable electroosmotic flow, enabling about 100 runs without requiring polybrene supplementation to the run buffer. The limits of detection were below 1 ng/mL for standard dyes, corresponding to a ~200-fold sensitivity enhancement. The integrated blotting–LVSEP–NACE method enabled high-resolution analysis of fine ink features, including individual strokes and dots as small as ~0.5 mm, and was successfully applied to the forensic examination of real-world documents. This approach offers a robust, nondestructive analytical tool for chemical analysis of original writings, with significant implications for forensic science, cultural heritage preservation, and archival research.

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Epitachophoresis for purification and concentration of biopolymers from large sample volumes

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Most bioanalytical applications require sample pre-separation. Non-affinity-based separation methods such as electrophoresis take advantage of differences in electro-migration to separate and concentrate selected analytes from crude samples. Recently, we have introduced epitachophoresis as a new electrophoretic technique for the concentration and separation of nucleic acids from milliliter sample volumes.

This communication reports on new instrumental systems for processing the crude samples by discontinuous electrophoresis in a circular arrangement – epitachophoresis (ETP) with almost unlimited concentration factor. Several experimental parameters have been studied, including the size, shape, and size of the zone stabilizing media and devices for large samples. Polyacrylamide or agarose gels are the most frequently used sieving and stabilizing media in slab gel electrophoresis; however, such sieving materials limit the size of the concentrated nucleic acids. In part of this work, we have also explored large pore materials and 3D printing to form rigid stabilizing manifolds to minimize liquid flow during the epitachophoresis. The device was printed using the stereolithography technique from a low water-absorbing resin. Different geometries of the 3D printed stabilizing manifolds were tested to concentrate ionic sample components in the anionic or cationic mode. Depending on its geometry, the devices can focus analytes from 1 to 50 ml of the sample into the collection cup with a size of 150 µL or less. Depending on the stabilization media and power used, the concentration time ranges from minutes to one hour. We have used the ETP to isolate DNA and RNA from biologically relevant samples in a single run, including formalin-fixed paraffin-embedded tissue. While the system was initially designed for extraction and focusing of nucleic acids, this presentation will also discuss the potential of the ETP for the separation and concentration of other analytes, including peptides and proteins.

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Portable Chip Electrophoresis Sensing for Biomedical Assay Based Moving Reaction Boundary

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Abstract: Recently, the concepts of electrophoresis titration (ET) chips were developed based on moving reaction boundary (MRB), including the ET of protein in milk sample forprotein contentassay,[1-3] the ET of enzyme catalysis for assays of enzymatic dynamics[4,5] and the ET of photocatalysis for melamineassay.[6] Herein, the two ET models of ELISA and uric acid were advanced based on the previous work.[1-6]In the first model,the ET was proposed for constructing ELISA chip of POCT just with a sextuplet electrode pairs and laminated cells. The chip had an anodic well, bridge channel, middle well, titration channel and cathode well in order. ELISA process was conducted in the bottom of middle well, where HRP catalyzed TMB as blue dimer of TMB^+. Under an electrical field of 29 V, the TMB dimer migrated into the titration channel and reacted with the ascorbic acid, creating a MRB. The MRB motion was a function of antigen content, indicating a visual ELISA. As a proof of concept, C-reactive protein was chosen as a model antigen. The experiments validated the ET ELISA model and method. Particularly, the chip was detector-free, power supply-free and sulfuric acid-free, making the method extremely simple, portable and safe. The method has potential to visual & portable ELISA in clinic, environmental and food safety immunoassay.

In the second model, uricase catalyzes UA in the anode well to allantoin, producing H_2O_2, which oxidizes colorless luceocrystal violet dye without charge as crystal violet (CV+) with one positive charge. Under an electric field, a MRB was created between the violet CV+ moving from the anode well into the channel and the alkaline sodium acetate in channel, resulting in an ET sensing. The model indicated that the distance of MRB under given conditions was as a function of UA content, implying an extraordinary simple sensing for UA. Based on the model, a series of experiments were conducted. The results evidently validated the model and method of ET-MRB. The experiments not only demonstrated the high facility and portability of MRB-ET model, but also showed the visuality, selectivity and rapidity. In addition, the experiments showed the sensitivity (< 0.1 mM), linearity (0.1-4.0 mM, $r^2 = 0.9948$), recovery (85~106%) and stability (RSD 3.8-7.1%). Finally, the developed method was successfully used for the determination of UA in urine and blood samples. All these results manifested the simple, portable and visual sensing of UA, and implied the potential of MRB-ET method to real POCT assay of UA in urine and blood samples.

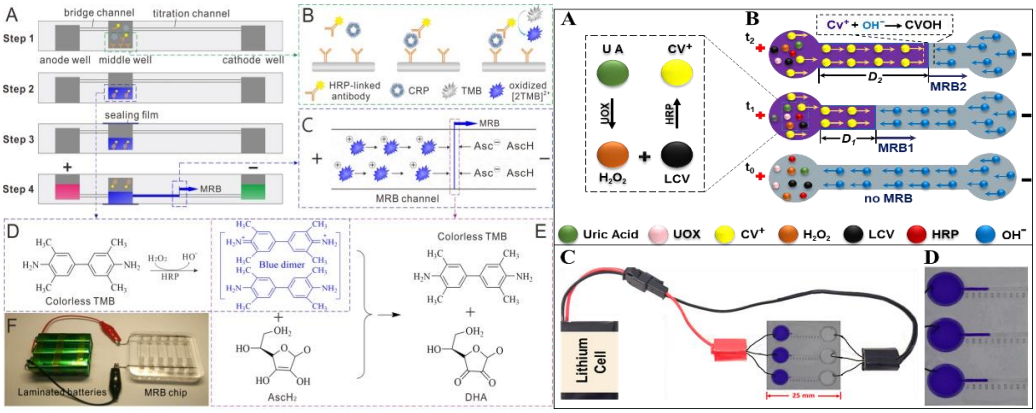

Figure 1. Left: ET model for ELISA of POCT. (**A**) ET ELISA assay in ET chip; (**B**) ELISA catalysisreaction in the middle well without use of H_2SO_4; (**C**) MRB formation between blue TMB dimer and ascorbic acid; (**D**) TMB substrates catalyzed by HRPenzyme as blue TMB dimer; (**E**) blue TMB dimer reduced by ascorbic acid as colorless TMB; (**F**) photograph of ET ELISA chip. **Right:** ET chip model for UA assay. (**A**)Uricase inducedcatalysis reaction of UA to H_2O_2 which further reacts with LCV in the presence of HRP forming CV^+; (**B**) MRB created with basic buffer OH^- and alkali CV^+; (**C**) Lithium cell providing electric field for ET; (**D**) Photo of a single MRB-ET run via an iPhone.

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Microfluidic Chip Combined with Mass Spectrometer for Single Cell Analysis

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Abstract

The micron-scale space of the microfluidic chips can be well matched to the cell size, which can easily simulate and precisely manipulate the cell growth microenvironment, and is an ideal platform for studying cell-cell interactions, signal transduction and communication, and cellular drug metabolism and delivery. Our research group has carried out research related to the development and application of microfluidic chip and mass spectrometry [1-3], successfully developed the world's first microfluidic chip mass spectrometry analysis device, and industrialized the technology transfer enterprises. This report mainly introduces the development of microfluidic chip combined with mass spectrometry for single cell analysis [4-6].

***Keywords**:* Microfluidics, Mass Spectrometry, Single cell analysis

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Biography

Dr. Jin-Ming Lin is a professor of Tsinghua University. From 1992 to 2002, He had studied and worked at Showa University and Tokyo Metropolitan University since 1992-2002. In 2004, he was appointed as a professor at the Department of Chemistry, Tsinghua University. In 2008, he was selected as ChangJiang Scholar Professor of the Ministry of Education, China. His main research interesting: 1) Microfluidic chip combined with mass spectrometry for cell analysis; 2) Research on rapid detection methods of foodborne pathogenic bacteria; 3) Research on the preparation method of air negative ions and its mechanism on promoting health. He is currently the deputy director of the Committee of Analytical Chemistry of the Chinese Chemical Society, the president of the Microfluidic System Branch of the Chinese Biophysical Society, the executive director of the Chinese Association of Analysis and Testing, the vice chairman of the Micro-nano Fluidics Technology Branch in Chinese Micro and Nanotechnology Society, the contributing editor of Trends in Analytical Chemistry, Associate Editors of J. Pharm. Anal., Chinese Chemical Letters.

Microfluidics for Extracellular Vesicles: from diagnosis to therapy

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Cancer is a leading cause of global mortality. Extracellular vesicles (EVs) play a crucial role in the occurrence and progression of cancer. In this presentation, we introduced our recent advancements on microfluidics-based EVs, new tools for EVs isolation, detection, and clinic diagnosis, prognosis, therapy as well. At first, we would discuss some our developed microfluidic-base methods for EVs analysis, and their applications for clinics, particularly with help of artificial intelligence. Then, clinical utilization of EVs as drug carriers for tumor therapy were investigated. We modified EVs by chemical modification and designed microfluidic chips and biomimetic particles for rapid and efficient isolation of EVs for tumor target and drugs delivery, which provides a new idea for the precise treatment of cancers. Finally, future prospective would be discussed.

Keywords: Extracellular Vesicles, microfluidics, diagnosis, therapy

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Lipid-Binding Peptides for Size-Selective Exosome Isolation and Molecular Profiling in Neurodegenerative Disease Liquid Biopsy

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Exosomes, characterized by high membrane curvature, lipid-packing defects, and carrying a diverse array of biomolecules, have garnered significant attention. High-performance isolation of exosomes as a promising liquid biopsy target is of great importance for both fundamental research and clinical applications. However, this is challenged by the prevalent heterogeneity of exosomes and the highly complex nature of biosamples. Here, we introduce a dual-targeting strategy based on the identification of a cationic peptide that can specifically recognize and bind phosphatidylserine (PS) exposed on the outer leaflet of exosomal membranes for the efficient isolation of exosomes from diverse biofluids. Apart from lipid recognition, we further demonstrated that the peptide can sensitively respond to membrane curvature, thus vesicle size in a PS-dependent manner. Leveraging synergistic effect of PS targeting and curvature sensitivity, polymer-based peptide-functionalized affinity separation materials were constructed with tailored molecular interactions toward lipid membrane of exosomes. High yield and purity for targeted fishing of exosomes in complex samples including cell culture medium, urine, and serum were achieved. The rapid and efficient isolation of exosomes from patient biofluids enabled downstream analysis of misfolded proteins as neurodegenerative markers, thereby differentiating healthy individuals, patients with mild cognitive impairment, and those with Alzheimer's disease. Moreover, integration of peptide-affinity capture with mass spectrometry–based proteomic profiling revealed differentially expressed exosomal proteins, highlighting their potential as theranostic targets for neurodegenerative disorders.

Keywords: affinity separation, peptides, lipid membrane, exosome, biofluids

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Advanced Molecularly Imprinted Polymers for Sample Pretreatment and Disease Diagnosis

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Abstract

Molecularly Imprinted Polymers (MIPs), as mimics of antibodies, are fabricated via template-directed polymerization, creating three-dimensional polymeric matrices that form tailored molecular recognition cavities after template removal. These imprints enable specific rebinding to original templates, generating antibody-like affinity and specificity. Particularly, MIPs can provide specificity that antibodies often fail to provide, such as the recognition toward glycans. Moreover, MIPs can be fabricated into nanoscale or incorporated with nanocores of diverse properties and functions, endowing nanoscale MIPs (nanoMIPs) with unique potential in many challenging applications such as separation, sensing, imaging, disease diagnosis, cancer therapy and viral inhibition. In this talk, the speaker will present the recent progresses of his team in the development of advanced MIPs and their applications in sample pretreatment and disease diagnosis.

New Methods Contributing to Metabolomics Analyses of Single Cells

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Metabolomics analyses of tissues, body fluids and cells are playing an increasingly important role in various fields of life science. For traditional cell metabolomics, millions of cells are generally required. However, in some special cases, such as stem cells, circulating tumor cells, primary cells and so on, the number of cells is very limited, and conventional metabolomics methods run into problems. It is necessary to develop novel methods for a small number of cells, even for single cell analysis. Although single cell is analyzed, to achieve statistically significant results, it is necessary to analyze a large number of cells under physiological conditions. Therefore, high-throughput single-cell analysis methods are required.

Over the past years, we have concentrated on the development of mass spectrometry (MS)-based single cells analysis techniques including capillary microsampling combined with high-resolution spectral stitching nanoelectrospray ionization direct-infusion MS, laser capture microdissection (LCM)-sample micromanipulation-MS, inertial microfluidics and pulsed electric field-induced ESI-MS, and concentric nanoESI-APCI hybrid source.

In this lecture our recent advancements on the single cells-related analysis methods for metabolomics and heterogeneity studies will be reported. The related work can be used not only for tens of cells or single cell metabolomics, but also for spatially resolved metabolomics study coupled with LCM.

Keywords: Single cell, Metabolomics, Mass spectrometry

Dynamic single-cell metabolomics platform and its application in cell-cell interaction

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Cellular heterogeneity plays an important role in many key biological processes such as tumor, aging, immunity and development, etc. From the perspective of metabolites, single-cell metabolomics helps to reveal the precise life activities and physiological states of individual cells, and achieve the analysis of cell heterogeneity and interactions in complex microenvironments of tissues [1]. Based on the single cell work in our group, for example, single cell organic mass spectrometry [2] and in-depth organic mass cytometry [3], a dynamic single-cell metabolomics platform and an automated single-cell dynamic metabolomic data analysis platform were constructed. A total of 40 isotopic labeled metabolites were traced in single cells, disclosing the heterogeneity of metabolic activity among single cells. A linear neural network machine learning model based on metabolic features was successfully established for binary classification of tumor cells and macrophages. The analysis results not only disclosed the metabolic alterations of the two interactions but also unveiled the heterogeneity of macrophage differentiation in the tumor microenvironment [4].

Keywords: Single cell, Metabolomics, Mass cytometry, cell-cell interaction

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Preparation of cation-exchange stationary phases for rare earth ion separation

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Abstract

Rare earth elements have unique optical, magnetic and electrochemical properties, and have been widely used. However, it is complicated to be separated and to get a single high-purity rare earth element [1]. Ion-exchange chromatography is a useful technology that can be used for rare earth separation.

In this presentation, several cation-exchange chromatographic stationary phases were designed and synthesized, and a variety of rare earth elements were completely baseline separated [2-4]. Further experiments show that these new kinds of facile chromatographic stationary phases have good repeatability and stability, and is expected to be used for separation and analysis of real rare earth samples.

Keywords: HPLC; Stationary phase; Chromatographic separation; Rare earth ions.

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Biography

Hongdeng Qiu received his PhD from Lanzhou Institute of Chemical Physics (2008). He did his postdoctoral research in Kumamoto University from 2009-2012 (JSPS Fellow). He worked in Lanzhou Institute of Chemical Physics in 2012-2024. Currently he is a professor in Ganjiang Innovation Academy, Chinese Academy of Sciences. His research interests are the applications of new materials in chromatography and sample preparation, especially for the separation and analysis of rare earth ions. He has published more than 260 papers and 50 patents. He is one of the chief-editor of Chinese Chemical Letters, and he is the Editorial Board Member of Chromatographia, Separation Science Plus, Chinese Journal of Chromatography and Progress in Chemistry.

Multi-Dimensional Characterization of Environmental Nanoparticles by Mass Spectrometry Techniques

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Characterization at nanoscale plays a crucial role in in-depth understanding the nature and processes of environmental nanoparticles (NPs). However, currently available characterization techniques suffer from many limitations, like lack of accurate molecular information, inability to real-time monitor intermediates, or vulnerability to sample matrix interference. To probe NPs in the environment, we developed a series of mass spectrometry-based techniques to analyze and characterize NPs. Specifically, we developed a chemical multi-fingerprinting platform (integrating elemental fingerprinting, high-resolution structural fingerprinting, and natural isotopic fingerprinting) for particle characterization and source tracing in environmental and biological samples. We found that exogenous NPs are widely present in the blood samples with extreme diversity in chemical species, concentration, and morphology. Furthermore, we have also developed a detection platform for airborne magnetic NPs and soot particles in complex media based on different MS techniques. These methods will greatly rich the toolbox of nanotoxicological research and nanomaterial risk assessment.

Keywords: mass spectrometry, nanoparticle, environmental analysis, stable isotope, source tracing

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Taylor Dispersion Analysis for Size Characterization of Charged Polymers and Silica Nanoparticles

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Abstract

Taylor dispersion analysis (TDA) is an absolute method that allows the determination of the hydrodynamic radius (RH) and molecular diffusion coefficient (D) of a solute by analyzing peak broadening in a laminar Poiseuille flow. TDA is normally used for size analysis of UV-absorbing or derivatized compounds using a commercial capillary electrophoresis (CE)-UV instrumentation. In this work, we investigate the capability of TDA for non-UV absorbing charged polymers and nanoparticles. A capacitively coupled contactless conductivity detector (C4D) was employed as a detector inline equipped with CE-UV instrumentation. Another application is to size the silica nanoparticles using the concept of light scattering using the equipped UV detector in CE instrumentation. Detection sensitivity is carried out using a continuous flow as 'frontal mode' and the sizing is achieved by using a hydrodynamic injection as in the 'plug injection mode' under the suitable eluent and TDA regime. Comparison of sizes obtained from the proposed alternative detection to the nominal size will be discussed.

Keywords: Taylor dispersion analysis; taylorgrams; light scattering; silica nanoparticle

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Chemical proteomic exploration of intercellular signaling

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Abstract:
Cell-cell interactions in tumor microenvironment are often mediated by ligand proteins secreted by one cell that are recognized by specific receptors presenting on neighboring cells. This intercellular communication activates signaling pathways through the induction of dynamic posttranslational modifications such as phosphorylation and protein-protein interactions. Mass spectrometry-based proteomics have been proven to be a robust approach for characterizing these signaling events on a global scale. However, proteomic studies of intercellular signaling directly from living systems have been challenging. In this talk, I will present our recent development of a series of chemical proteomic approaches toward this end, especially for new proximity labeling approaches. Beside our recent discovery of LIF as the new drug target and potential biomarker for pancreatic cancer, our work explored many new secreted ligand-membrane receptor pairs and provided valuable resources for systematically understanding dysfunctional signaling networks in pancreatic tumor microenvironment in a context closer to physiological condition than was previously possible.

Superior Selectivity of Copper Single-Atom Nanozyme Mimicking Galactose Oxidase

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Since some natural enzymes are expensive and unstable, nanozymes—nanomaterials that mimic enzymes—have been created. Notably metal-centered nanozymes known as single-atom nanozymes resemble the structure of naturally occurring enzymes based on metals. In order to replicate the activity of natural galactose oxidase, Cu-N-C single-atom nanozyme (SAN) is created here with superior peroxidase- and increased oxidase-like capabilities. By oxidizing D-galactose and primary alcohol but not L-galactose or other carbohydrates, Cu-SAN exhibits stereospecific activity similar to that of natural galactose oxidase. When galactose is exposed to oxygen, the SAN can catalyze its oxidation, resulting in the production of hydrogen peroxide as a byproduct. After being catalyzed by the SAN, the generated hydrogen peroxide oxidizes 3,3′,5,5′-tetramethylbenzidine to form the characteristic blue product. With a detection limit as low as 0.23 μM, the absorbance and galactose concentration have a linear relationship in the 1–60 μM range. This method can be used to identify galactose in some dairy products and other commercial products, as well as to diagnose galactosemia condition.

From Bottles to Bodies: High-Resolution Microplastics Detection Using Laser Direct Infrared in Malaysia

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Abstract

Microplastics (MPs) have become a growing environmental and health concern due to their persistence, ubiquity, and potential biological effects. Leveraging the state-of-the-art Laser Direct Infrared (LDIR) chemical imaging spectroscopy, this study provides the first integrated assessment of MPs contamination in Malaysia across multiple exposure pathways [1]. Analysis of bottled water revealed the widespread presence of MPs, predominantly smaller than 50 µm, with a diverse range of polymer types detected, including natural polyamide, cellulose, chitin, rubber, polyamide (PA), and polyethylene terephthalate (PET) (Fig. 1). Almost all of the EU's priority MPs were identified [2], with PA, PET, polyethylene (PE), polypropylene (PP), and polyurethane (PU) being among the most common. Extending the scope, carbonated and isotonic beverages packaged in PET bottles, aluminium cans, and laminated cartons were examined, showing marked differences in MPs abundance and composition depending on the packaging material, highlighting packaging as a key factor influencing contamination. Finally, MPs in human fecal samples were analysed from healthy individuals and patients with colorectal disease, revealing distinct differences in polymer types, size distributions, and patterns between the groups, suggesting possible links between MPs exposure and gastrointestinal health. These findings demonstrate the versatility and precision of LDIR in detecting and characterising MPs across diverse matrices, underscore the impact of beverage packaging on contamination levels, and point to the urgent need for further research into the health implications of chronic MPs exposure.

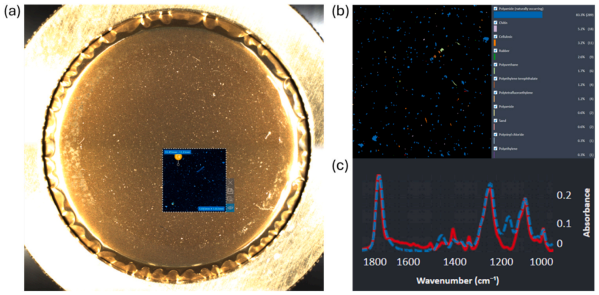

Fig. 1. (a) Gold-coated membrane with particles from a bottled water sample (F-U), showing a 5 mm × 5 mm scanned area (black square) on the 15 mm membrane, (b) IR image of detected particles coloured by polymer type, and (c) LDIR spectra of identified MPs (blue: reference; red: measured).

Keywords: Microplastics, Laser Direct Infrared, drinking water, colorectal disease

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Multi-organoid Microphysiological Systems and Their Applications in Pharmacology and Toxicology Research

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Abstract body (up to 350 words)

Multi-organ-on-a-chip technology demonstrates significant potential in disease modeling, drug screening, and precision medicine due to its ability to simulate multi-organ interactions. Theoretically, simulated blood flow within microfluidic chips should ensure adequate interactions among organoids. However, in practical organ-on-a-chip applications, inter-organoid interactions are not always observable. Furthermore, variations in interaction intensity between identical organoid combinations on chips are often attributed to functional differences in organoids, though whether this is the sole cause remains unclear.

To address this issue, the presenter's team developed a microphysiological system incorporating nine interconnected organoids. Through a series of experiments, they comprehensively investigated how microfluidic chip design—including spatial arrangement, chip parameters, functional detection, and communication media—affects organoid interactions and their underlying patterns.

our study revealed that the spatial sequencing of organoids on the chip significantly alters their functionality and interaction patterns. Specifically, functional changes in target organoids are primarily influenced by the type of their upstream adjacent organoids, with this effect being independent of physical distance. Other chip parameters — such as flow rate, organoid chamber design, and simulated blood volume — non-linearly impact organoid interactions. Additionally, intact vascular barrier structures substantially influence inter-organ communication.

These findings establish new research directions for precisely simulating dynamic interactions at the human organ level. They will enhance the capability of multi-organ-on-a-chip systems to fulfill their potential in medical research and novel drug discovery.nd.

Keywords: organ-on-a-chip, multi-organ-on-chip, organ-interaction

Development of isotope dilution-liquid chromatography/tandem mass spectrometry (ID-LC/MS/MS) for the accurate determination of aflatoxins and vitamin B_{12} in food

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Certified reference materials (CRM) are crucial for disseminating national measurement standards in chemical metrology, ensuring the reliability of testing laboratories. As the National Metrology Institute of Korea, KRISS has been developing CRMs for food analysis. As a part of this effort, we need to develop higher-order reference methods using isotope dilution-liquid chromatography/tandem mass spectrometry (ID-LC/MS). In this talk, I will present two recent projects focused on analytical method development. Aflatoxins are highly toxic mycotoxins produced as secondary metabolites by various fungi. They frequently occur in a wide variety of foodstuffs, including cereals, coffee, and nuts. We have developed an accurate ID-LC/MS/MS method for simultaneous analysis of four aflatoxins in grains and coffee samples [1,2]. We optimized the sample preparation procedures for each food matrix and evaluated the matrix effects to eliminate the measurement bias. Next, I will discuss the analysis of Vitamin B_{12} analysis in infant formula. Vitamin B_{12} is an essential nutrient used for formation of red blood cells and DNA synthesis and cyanocobalamin has been employed for fortification of vitamin B_{12}. Due to the hygroscopic property of cyanocobalamin, we used quantitative NMR to determine the concentrations of standard solutions. The sample preparation procedure, including cyanidation and extraction conditions, was optimized. The developed methods were validated by evaluating their performance and subsequently applied to samples obtained from local markets.

Keywords: Isotope dilution-mass spectrometry, aflatoxins, vitamin B_{12}, validation

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Portable Sensors Utilizing Small Sample Volumes for Forensic and Security Applications

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This presentation highlights recent research on the development of portable sensors for on-site chemical analysis using small sample volumes. Emphasis is placed on low-cost, disposable platforms fabricated from accessible materials such as paper, pencil leads, and gold leaf, enabling both electrochemical and colorimetric detection. The target applications focus on forensic and security-related scenarios, particularly in field-based or resource-limited settings. Demonstrated devices include: (i) a microfluidic paper-based analytical device (μPAD) [1,2] and a gold leaf-based electrochemical sensor [3] for gunshot residue (GSR) analysis, including the estimation of shooting distance, and (ii) a pencil-based voltammetric sensor for the detection of 2,4,6-trinitrotoluene (TNT), integrated with drone-assisted sampling [4]. These examples illustrate practical strategies for deploying portable, low-volume chemical sensing platforms to support rapid forensic and security screening beyond conventional laboratory environments.

***Keywords*:** Paper-based, electrochemical sensor, gunshot residue, pencil, drone, explosives

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Portable Capillary Electrophoresis Instrument for On-Site Forensic Analysis

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The field portable instrument was designed in our laboratory for ease of construction and can be assembled with limited mechanical and electronic manufacturing required. It is contained in a robust Peli protector case with the approximate dimensions of 40 x 33 x 17 cm and a weight of 8 kg. Polarity switching allows the determination of either cations or anions by capillary zone electrophoresis. For ease of use and high reproducibility, the injection of the sample was automated. The electropherograms are obtained with a universal contactless conductivity detector. This is of a differential design, which features automatic background correction to facilitate its use and suppression of drifts. Control of the instrument and data acquisition is achieved by attaching a notebook computer. The use of the instrument was successfully demonstrated for two forensic applications: the determination of the date rape drug gamma-hydroxybutyrate (GHB) in beverages and in urine, and for the estimation of the post-mortem interval (PMI) by determining the potassium concentration in the vitreous humour.

Keywords: capillary zone electrophoresis, contactless conductivity detection, portable instrument, forensic analysis

Ion-pairing in peptide RPLC separations: unexpected features and their consequences

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Reversed-phase HPLC is the most popular method for peptide separations applied in various formats from preparative scale down to nano-flow applications in proteomics. While the dominant role of ion-pairing RPLC separation mechanism is widely acknowledged, its consequences for peptides separation selectivity are still poorly understood except for apparent differences in hydrophobicity of different ion-pairing additives (e.g. formic acid vs. trifluoroacetic acid).

Proteomic LC-MS technology provided separation scientists with virtually unlimited datasets to explore these selectivity features. First, we established a significant effect of ion-pairing environment on hydrophobicity of the residues adjacent to positively charged peptides' functional groups. This led to the development of the first position-dependent peptide retention time prediction models such as Sequence-Specific Retention Calculator [1]. Recently we explored peptide retentivity and selectivity features for the sorbents of different pore sizes and found a significant reduction of available surface area due to ion-pairing induced size exclusion for the sorbents in 60-200 Å pore size range [2]. Apparently, the size of hydrated counterions (acetate, formate, trifluoroacetate) is the major driver behind selectivity changes observed between RPLC packings of different pore sizes. It also determines variation in retention time shifts following peptides' chemical or post-translational modifications. This presentation reviews the ion-pairing driven selectivity changes in peptide RP HPLC, our 20-years progress in better understanding of the separation mechanism and outline future developments in designing optimal stationary phases for peptide separation.

Keywords: peptide RP HPLC, peptide retention time prediction, ion-pairing mechanism

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Noncovalent Fluorophore Labeling of Biotherapeutics in Sodium Dodecyl Sulfate Capillary Gel Electrophoresis

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Sodium dodecyl sulfate capillary gel electrophoresis is one of the frequently used methods for size-based protein separation in the biopharmaceutical industry. To increase throughput quite a few multicapillary electrophoresis systems have been recently developed but most of them only support fluorescent detection, requiring fluorophore labeling of the sample proteins. To avoid the time-consuming derivatization reaction, we developed an on-column labeling approach utilizing propidium iodide in SDS-CGE of proteins, a dye only used before for nucleic acid analysis. As a key ingredient of the gel-buffer system, the oppositely migrating positively charged propidium ligand *in migratio* complexes with the SDS-proteins, i.e., supports *in situ* labeling during the electrophoretic separation process, not requiring any extra pre- or post-column derivatization step. A theoretical treatment is given to shed light on the basic principles of this novel online labeling process. Considering the increasing number of protein therapeutics on the market next, we focused on the labeling optimization of a therapeutic monoclonal antibody and its subunits, including the addition of the non-glycosylated heavy chain. Peak efficiency and resolution were compared between non-covalent and covalent labeling. The effect of ligand concentration on the effective and apparent electrophoretic mobility, the resulting peak area, and resolution were all evaluated in view of the theoretical considerations. In addition, the effects of the three most important user-adjustable operational parameters (temperature, gel concentration, and electric field strength) were also investigated on the electrophoretic mobility and resolution of SDS-protein complexes in the presence of propidium iodide in the gel-buffer system. Our results underline the importance of optimizing these key parameters in SDS-CGE with propidium-mediated LIF detection to obtain rapid and high-resolution separation of complex protein samples such as biopharmaceuticals.

Novel Approaches in Surface-Enhanced Raman Spectrometry for Analysis in Complex Matrices

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Surface-enhanced Raman spectrometry (SERS) is a powerful analytical technique that overcomes the low sensitivity of Raman scattering by amplifying the signal of analytes near metal nanostructures. This phenomenon opens new possibilities for applications, especially for tracing low-abundance compounds.

We developed a nanospray-assisted deposition method for the precise and homogeneous application of silver nanoparticles onto hydrophilic nanofibrous layers. This controlled deposition process enabled the detection of a biologically important peptide otherwise undetectable by conventional Raman spectrometry.[1] However, a significant challenge in analyzing real samples is matrix interference, which complicates SERS spectra interpretation.

To address this, we initiated the development of a hyphenated system coupling SERS with capillary electrophoresis (CE).[2] Our early-stage work involves designing etched-glass microfluidic chips that allow introducing colloidal silver nanoparticles downstream from the separation capillary. This configuration is intended to prevent nanoparticle interference with the separation process while enabling real-time SERS detection. To further enhance SERS performance and stability, we are also developing advanced nanomaterials to maintain colloidal stability and SERS sensitivity in high-ionic-strength solutions.[3] Furthermore, we are exploring composite nanostructures for the targeted analysis of specific compound classes.[4]

Keywords: Raman spectrometry, nanostructures, separation, matrix, deposition

Acknowledgement

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Developing Ambient Ionization Mass Spectrometry Strategies and Their Applications in Comprehensive Natural Medicine Analysis

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Ambient ionization mass spectrometry (AIMS) enables direct, open-air ionization with virtually no sample preparation, offering a rapid and versatile analytical window for complex matrices. Leveraging representative modalities such as direct analysis in real time (DART), desorption electrospray ionization (DESI) and liquid-extraction surface analysis (LESA), our group has established a suite of workflows targeting the characteristic active or toxic constituents of traditional Chinese medicine (TCM). DART-MS supports straightforward screening of hepatotoxic pyrrolizidine alkaloids in herbal decoctions, finished preparations and related food products; Laser-assisted thin-layer chromatography (LA-TLC) coupled to DART and TLC spray-ionization platforms extends coverage to alkaloids, flavonoids, anthraquinones, volatile oils and organic acids and enables in-situ distribution mapping of triterpenic acids in *Poria cocos*. LESA-MS provides non-destructive surface fingerprints for high-value materials such as *Panax notoginseng* and agarwood, while a chip-based nano-ESI interface facilitates high-throughput authentication of bear-bile preparations and screening of natural enzyme inhibitors. To translate these laboratory capabilities to point-of-need contexts, we have integrated paper capillary spray with a handheld mini-MS. This portable platform delivers rapid chemical fingerprints that distinguish and grade agarwood, fragrant rosewood and aged Citrus peels, and simultaneously monitors pyrrolizidine alkaloids, thereby supporting authenticity verification, safety assessment and quality benchmarking outside the laboratory. Together, these complementary AIMS strategies create a coherent laboratory-to-field continuum that aligns with green-chemistry principles and modern regulatory expectations, positioning AIMS as a practical, sustainable solution for comprehensive quality evaluation and mechanistic exploration of natural medicines.

Keywords: Ambient Ionization, mass Spectrometry, natural Medicine, traditional Chinese medicine, quality evaluation

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Isomer-Specific Separation and Glycomic Insights into Spatiotemporal Dynamics of the Mammalian Brain

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Liquid chromatography–mass spectrometry (LC–MS) provides powerful capabilities for resolving glycan and glycolipid heterogeneity, yet isomer separation has remained a major challenge in brain glycosylation research. Here, we present separation-based workflows enabling isomer-specific characterization to uncover spatial and temporal dynamics of the mammalian brain glycome. Using ultra-high-performance LC coupled with tandem MS, we achieved baseline separation of intact ganglioside isomers, including GD1a and GD1b, and profiled their region-specific distribution across nine mouse brain areas. This isomer-specific approach revealed conserved core structures, differential abundance of a- and b-series gangliosides, and distinct O-acetylation features, emphasizing the functional relevance of isomer separation in molecular neuroscience. Complementary glycomic profiling was performed using porous graphitized carbon nano-LC/MS for N-glycans in mouse and human brains. Separation-based analysis demonstrated that while a conserved glycan core is maintained, extensive developmental remodeling occurs, particularly in the prefrontal cortex. Sialylated and fucosylated glycans emerged as age-dependent markers, and a synthetic glycosylation map delineated pathways underlying maturation processes. Together, these studies establish chromatographic isomer separation as a critical foundation for decoding brain glycosylation. By integrating isomer-specific separation with LC–MS, we provide a systems-level view of glycan and glycolipid organization that captures both spatial heterogeneity and developmental plasticity. These findings underscore the central role of separation science in advancing glycomics and highlight its potential for identifying therapeutic targets in neurodevelopmental and neurodegenerative disorders.

Keywords: Isomer separation, LC–MS, glycome, glycolipids, brain

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Advancing Food Safety from Bench to Field: Portable Electrochemical Devices with In-Situ Sample Pretreatment for Sulfite Detection

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Ensuring food safety remains a critical global challenge, particularly in products prone to chemical preservative abuse, such as frozen shrimp. Although sulfite preservatives are effective in preventing spoilage, their use must be strictly controlled due to potential health hazards. We will present the translational journey from laboratory-based chemical analysis to the development of a compact, user-friendly bench-top instrument that incorporates a unique electrochemical sensor for quantifying sulfite residue in frozen shrimp and other types of seafood. The electrochemical sensor operates in the gas phase above the closed vessel containing the sample. The sensor measures the sulfur dioxide gas generated from the sulfite in an aqueous suspension within a sealed extraction chamber. This modular device integrates an extraction unit with a reusable electrochemical sensor mounted in the lid, capable of providing consistent readings for at least 60 consecutive measurements. The system is electronically controlled and integrated with a software platform that enables fine-tuning of electrochemical parameters, signal monitoring, and real-time reporting sulfite concentration on a graphical display. Our instrument not only delivers a useful quality control tool for the frozen food industry but also serves as a product for a local start-up entrepreneur to explore the commercialization of this sensor innovation.

Keywords: sulfite, electrochemistry, sensor, gas phase detection, innovation, food safety