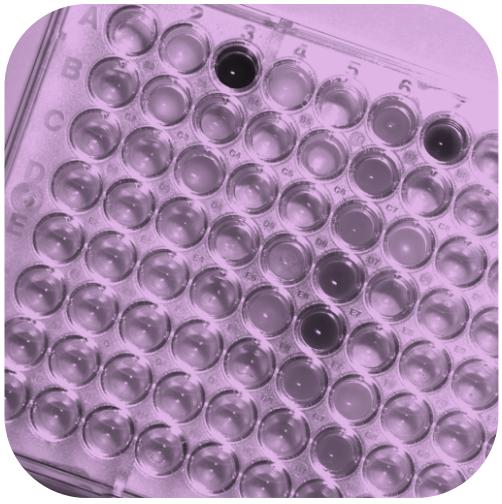
*CH*analysis 2015

Meeting of Swiss Analytical Scientists

April 10–11, 2015
Dorint Hotel Beatenberg

Organized by the Division Analytical Sciences
of the Swiss Chemical Society



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Analytical Sciences

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General Information

Organizing Committee:

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Ralph Schlapbach, Universität Zürich, ETH Zürich
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Participation fee (includes meals and accommodation)

Regular fee: CHF 300.–
Students including PhD students: CHF 100.–

Scientific program

Friday, April 10, 2015

12.00 Lunch

14.00 Opening, **Marc Suter**, Eawag Dübendorf

14.10 **Kirstin Schirmer**, Eawag Dübendorf
Analytical tools in ecotoxicology

15.00 **Session 1: Labeling Strategies**
Stefan Schürch, University of Bern
Moderator

14.45 **Bernd Bodenmiller**, University of Zürich
Analysis of single cells through time and space by mass cytometry

15.30 Coffee break

16.00 **Tobias Bruderer**, University of Geneva
Creation of a high-quality MS metabolomics library to identify human metabolites in urine by LC-SWATH-MS analysis

16.15 **Anina Thurnheer**, Zurich University of Applied Sciences
Mass spectrometry-based glycan analysis of the glycoprotein ricin

16.30 **Aleksandra V. Zabuga**, EPFL
Photofragmentation mechanism of peptides in the gas phase

16.45 Discussion

18.00 Dinner

19.30 Poster session

Saturday, April 11, 2015

9.00 **Session 2: Imaging**

9.00 **Gérard Hopfgartner**, Université de Gèneve
Moderator

9.00 **Markus Stöckli**, Novartis Pharma AG, Basel
About MS imaging and its impact in biomedical research

9.30 **Andras Kiss**, Université de Gèneve
Effects of sample preparation and instrument parameters on the quantitative performance of SRM mass spectrometry imaging

9.45 **Yang Yue**, Eawag Dübendorf
Cellular uptake of citrate-coated silver nanoparticles in RTgill-W1 cells

10.00 **Bodo Hattendorf**, ETH Zürich
First ^{14}C-scans on carbonated records by laser ablation - AMS

10.15 Discussion

10.45 Coffee Break

11.15 **Session 3: Small Molecules**
Laurent Bigler, University of Zürich
Moderator

11.15 **Stefan König**, University of Bern
Screening of forensic and clinical samples: from immunoassay tests to SWATH acquisition

11.45 **Lukas Bregy**, ETH Zürich
Differentiation of dental bacteria in in vitro *cultures and human saliva by secondary electrospray ionization mass spectrometry*

12.00 **Peter C. Hauser**, University of Basel
Capillary electrophoresis for on-site analysis

12.15 **Jingying Zhai**, University of Geneva
Ion-selective nanospheres as heterogeneous indicator reagents in complexometric titrations

12.30 Discussion

13.00 Lunch

14.00 End of the meeting

Abstracts

Analytical tools in Ecotoxicology

Kristin Schirmer

Head of Department - Environmental Toxicology
Eawag, Swiss Federal Institute of Aquatic Science and Technology

Marc keeps telling me that I am the maverick (the eccentric?) of the *CH*analysis conference. I wonder – is this good or bad? As an ecotoxicologist, I strive to understand how chemicals impact on organisms in the environment – wait a second – chemicals? So can an ecotoxicologist live without analytical tools? Can an ecotoxicologist do without knowing how much of a certain chemical is available to an organism, where it ends up in the organism or also what an organism can do to it? Obviously not, although I agree that there is still way too little analytical chemistry in ecotoxicology. But why? In my talk, I will provide insights into the application of analytical tools from the eccentric ecotoxicologist's point of view and highlight difficulties and needs that we face with regard to chemical quantification and visualization in the complex matrixes and structures that we deal with.

Analysis of single cells through time and space by mass cytometry

Bernd Bodenmiller
Institute of Molecular Life Sciences, University of Zürich

Introduction

Tumors consist of heterogeneous cancer and normal cells that communicate with each other in tumor microenvironments (TME). This communication drives tumor progression, metastasis formation and drug resistance. For an understanding of the processes in the TME, comprehensive investigation of its components and their relationship is necessary. This demands imaging approaches that can simultaneously measure dozens of biomarkers to define cell types, their functional and signaling states, and spatial relationships.

Methods

For highly multiplexed tissue imaging at subcellular resolution, we have coupled immunohistochemical (IHC) methods with high-resolution laser ablation and mass cytometry (MC) (1). In MC, metals are used as reporters on antibodies. Analysis of metal abundances using MC allows determination of biomarker expression. In the presented approach, tissue sections were stained with metal-tagged antibodies using IHC protocols. Antibodies were selected to target readouts relevant to breast cancer. Then the tissue was ablated spot by spot (2), and the ablated material was analyzed using a CyTOF MC instrument. After data preprocessing, 44 isotope signals were plotted as a high-dimensional image of the tissue. Single-cell features were segmented and the single cell marker expression data were extracted for downstream bioinformatics analyses.

Results

Imaging MC provides targeted, high-dimensional analysis of cell type and state at subcellular resolution. The imaging approach enables the simultaneous visualization of up to 120 proteins and their modifications. Application to breast cancer samples allowed delineation of cell subpopulations and cell-cell interactions, highlighting tumor and TME heterogeneity. As such it has the potential to yield insights of the TME by exploiting existing large collections of FFPE tumor samples and associated clinical information.

References:

(1) Giesen et al. Nat. Meth. 2014
(2) Wang et al. Anal. Chem. 2013.

Creation of a high-quality MS metabolomics library to identify human metabolites in urine by LC-SWATH-MS analysis

Tobias Bruderer,[1] Emmanuel Varesio,[1] Eva Duchoslav,[2] Lyle Burton,[2] Ron Bonner,[2] Gérard Hopfgartner[1]

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Introduction

Metabolomics studies try to understand biological changes by looking at differences in the occurrence of hundreds of observed metabolites. The correct identification of these small molecules based on LC/MS peaks is essential but remains a challenge. It relies on the comparison with reference standards that are often not easily obtainable for metabolites. The currently available MS/MS libraries are of varying quality and dependent on the acquisition instrument. Furthermore no information is available for relative MS response factors or retention factors. We generated a MS metabolomics library for 530 metabolites reported in the human metabolome database (HMDB) taking into account adduct MS response, fragment annotation and retention times and used it for the identification of human metabolites in urine.

Methods

High resolution MS/MS spectra for 530 metabolite standards were acquired at 16 different collision energies on a Triple TOF 5600 mass spectrometer by flow injection analysis. Retention times were determined by analyzing mixtures of 10 compounds on an Ulti Mate 3000 RSLC system. Urine samples were analyzed by UHPLC-MS using data independent acquisition (DIA) with SWATH (sequential window acquisition of all theoretical fragment ion spectra) acquisition mode for a mass range of m/z = 50-1000 with a collision energy spread of CE = 20-80 eV. Metabolites were identified with PeakView 2.2 with the MasterView 1.1 package and the LSMS metabolomics library v1.0.

Results

Our MS metabolomics database includes the TOF MS response factors for the various adducts such as $[M+H]^+$, $[M+NH_4]^+$, $[M+Na]^+$, $[M-H]^-$, $[M+CH_3COOH-H]^-$, $[M+Cl]^-$, dimers and losses of H_2O, CO_2 and NH_3. The distribution of precursor intensities showed a high dynamic range of 5 orders of magnitudes for protonated and deprotonated ions. Composite MS/MS spectra were generated based on individual MS/MS spectra acquired at 16 different collision energies (CE). Fragment structures were assigned to the composite spectra with in-house software. Only fragments with assigned structure were included in the library. The distribution of fragments at different CE was investigated. The highest number of unique fragments was observed at higher CE than expected. Urine samples were searched for the library compounds based on the known retention times for 349 compounds (ESI positive) respective 410 compounds (ESI negative mode). Compounds with good MS/MS matching were further investigated with a closer look at isobaric and co-eluting compounds. 110 unique compounds were identified by exact mass, retention time and MS/MS matching.

Conclusion We created a high-quality MS metabolomics library based on composite MS/MS spectra with annotated fragment structure and retention factors. This library provides the basis for identifying and quantitating these metabolites by LC-SWATH-MS in biological samples.

Mass spectrometry-based glycan analysis of the glycoprotein ricin

Anina Thurnheer,[1] Roland Josuran,[1] Christian Müller,[1] Marc-André Avondet,[2] Christiane Zaborosch[1]

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[2]Spiez Laboratory, Toxinology Group, Spiez

Ricin is a potent toxin from the plant *Ricinus communis* which is included on list 1 of the Chemica Weapons Convention because of its high toxicity and easy availability. Ricin is present as a 59 kDa glycoprotein in the seeds of *Ricinus communis* and consists of two disulphide-linked subunits. *N*-glycan species of ricin were identified by enzymatic cleavage from the protein part, separation of the glycan species by porous graphitized carbon chromatography (PGCC) and detection with an electrospray ionization quadrupole time-of-flight mass spectrometer (ESI-Q-TOF-MS). Glycosylation sites in the protein were identified by capillary electrophoresis separation of proteolytically digested glycopeptides coupled to a mass spectrometer (CE-MS). In addition, a CE method was established for the analysis of the monosaccharide composition of glycans with detection by means of LED-induced fluorescence (CE-LEDIF).

Ricin was shown to contain high-mannose type glycans with additional monosaccharides, such as xylose, and different linkages of monosaccharide building blocks compared to animal glycoproteins. Ten different high-mannose glycan species of ricin were identified using PGCC-MS analysis. Linkages of the monosaccharide building blocks of the glycans were further characterized by exoglycosidase digestion with α(1-2)-mannosidase and α(1-6)-mannosidase.

Photofragmentation mechanism of peptides in the gas phase

Aleksandra V. Zabuga, Michael Z. Kamrath, Oleg V. Boyarkin, and Thomas R. Rizzo

Laboratoire de chimie physique moléculaire, Ecole Polytechnique Fédérale de Lausanne, SB ISIC LCPM Station 6, CH-1015 Lausanne, Switzerland

Are peptides and proteins intrinsically photostable or do they require help from the surrounding molecules to protect themselves against the UV radiation? To answer this question we have isolated individual peptides in the gas phase, excited them with a UV laser, and monitored their structural changes prior to fragmentation.

When phenylalanine- or tyrosine-containing peptides absorb a UV photon, it is known that a portion of the molecules will fragment after the electronic energy converts to the vibrational energy in the ground electronic state. In this case one might expect to observe cleavage of the weakest bonds in the molecule, which are typically backbone cleavages. However, by warming the UV excited molecules further with an infrared laser, we observe that the dominant fragment results from phenylalanine/tyrosine side-chain loss, which cannot be explained by statistical fragmentation from the ground state.

By performing a series of time resolved UV-IR spectroscopic experiments and DFT calculations, we have determined that a part of the molecules gets trapped in a triplet state that can live for more than tens of milliseconds. Our findings have direct applications for increasing the sensitivity of photofragment spectroscopy and help further understand the UV photodamage of peptides.

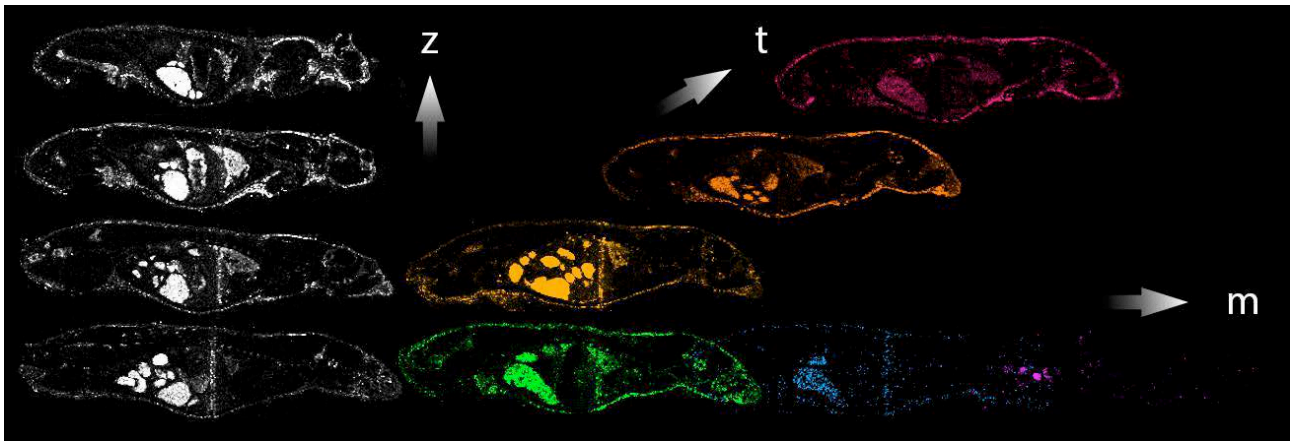
About MS imaging and its impact in biomedical research

Markus Stöckli

Analytical Sciences and Imaging, Novartis Institutes for BioMedical Research, Basel, Switzerland

At the beginning there were colorful images… MS Imaging came a long way and with the introduction of MALDI MSI in 1997 it started to have a significant impact on biomedical analytics. This presentation discusses the achievements of this enabling technology with a focus on specific applications of unique value for our search to find cures, but also currently unmet needs.

The image below summarizes the ability of MS imaging, showing distribution data of multiple analytes, measured directly from animal sections:



More info: http://www.maldi-msi.org

Effects of sample preparation and instrument parameters on the quantitative performance of SRM mass spectrometry imaging

Andras Kiss, Tiffany Porta,[a] Antoine Lesur,[b] Emmanuel Varesio, Gérard Hopfgartner

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In biomedical research the three most important questions are the identity, spatial distribution and quantity of unknown compounds. MALDI mass spectrometry imaging is a label free surface analysis technique that promises to be able to answer all three questions and as such it found widespread use in diverse fields such as biomarker discovery or pharmaceutical science in the recent years. In the field of mass spectrometry imaging one of the main focuses is on the development and improvement of imaging protocols to reliably quantify various compounds from complex surfaces. Despite the intensive research in this area, the influence of the instrumental parameters on the quantitative performance of MALDI mass spectrometry imaging is still poorly understood. Among those are the effects of different matrix deposition methods and the pixel size of the measured image on the precision of the quantitative data. The better understanding of these parameters would enable the development of better optimized quantitative methods for future applications.

In this work we present the evaluation of the novel iMatrixSpray device for the flexible and reproducible automated deposition of different matrices on surfaces in comparison with the traditional dried droplet matrix preparation method. Additionally, several instrumental parameters such as different stage speed settings, laser power and frequency and spatial binning of the final image were evaluated in order to minimize pixel-to-pixel variability of a standard compound's signal intensity. Our results suggest that pixel size has a great effect on the precision of MALDI imaging and spatial binning of at least 4-5 pixels is necessary in order to achieve reliable quantitation with a precision better than 10-15%. Finally, the performance of the iMatrixSpray device was demonstrated by the quantitative imaging of cocaine from coca leaf samples.

Cellular uptake of citrate-coated silver nanoparticles in RTgill-W1 cells

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[2]EPFL, Institute of Environmental Engineering, Lausanne, CH 1015, Switzerland
[3]ETHZ, Institute of Biogeochemistry and Pollutant Dynamics, Zurich, CH 8092, Switzerland

With the increasing application of silver nanoparticles (AgNP) in daily life, more attention is paid to the effects of AgNP on aquatic environments. In aqueous solutions, AgNP behaviour is affected by a variety of factors which lead to altered AgNP size and toxicity. This work aims to explore the cellular uptake of AgNP in RTgill-W1 cell line, which was derived from rainbow trout (Oncorhynchus mykiss) gill. Citrate coated AgNP (cit-AgNP, 20 nm, -30 mV) were used in this work.

In the previous work, AgNP showed particle-specific effect to RTgill-W1 cells exposed for 24 h. The lysosomal membrane integrity was the most sensitive endpoint and the cellular metabolic activity was the lest sensitive endpoint among all three cell viability measurements. On the contrary, this kind of difference was not observed in $AgNO_3$ exposures.

The cellular uptake of AgNP by RTgill-W1 cells was studied by inductively coupled plasma mass spectrometry (ICP-MS) and transmission electron microscopy (TEM). In the same external silver treatment, the internal silver concentration was 2.5-10 folds higher in AgNP exposures than in $AgNO_3$ exposures. Moreover, the silver accumulation reached to saturation in the high concentration AgNP exposures, which was not observed in $AgNO_3$ exposures. This means that the RTgill-W1 cells take up of AgNP and Ag+ via different routes. The transmission electron microscope and element analysis results showed that RTgill-W1 cells took up AgNP via an energy depend pathway and stored in endocytic compartments such as endosomes and lysosomes.

As in the cells, it is AgNP-protein corona that affect cell activity. Identification and studying these proteins adsorbing to AgNP surface will be very help to explain the mechanism of AgNP particle-specific effect to cells. The strategy of AgNP-protein corona separation was only lysing the intact subcellular organelles with AgNP, which could avoid proteins interference from other organelles and cytoplasma. Proteins in the AgNP-Protein corona was identified by nano-LC-MS/MS.

In summary, current work showed that AgNP could be taken up by fish gill cells and accumulated in endocytic compartments. Dozens of proteins adsorbed to AgNP. Linking to the observed cytotoxicity and the identified protein functions, an initial mechanism of AgNP toxicity was described in cell level.

First ^{14}C-scans on carbonated records by laser ablation - AMS

C. Welte,[1,2] B. Hattendorf,[1] L. Wacker,[2] M. Christl,[2] J. Koch,[1] H.-A. Synal,[2] D. Günther[1]

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The asymmetrical flow field-flow fractionation (AFlFFF) coupled on-line to UV- detection and inductively coupled plasma-mass spectrometry was used in the present study to quantify the size and the elemental distributions in a system containing carboxyl-terminal group polymer coated CdSe/ZnS quantum dots (QDs) as model engineered nanoparticles, Suwannee River fulvic (SRFA) or humic (SRHA) acids and trace metals (Cu or Pb). Obtained results showed a narrow QD size distribution with an average hydrodynamic diameter of 12.8 ± 0.5 nm. Addition of both 20 mg L^{-1} SRFA, or SRHA, and 100 µg L^{-1} or Cu and Pb did not significantly influence the QDs hydrodynamic size distributions. The AFlFFF-UV-ICP-MS set up also offers considerable potential for characterization of the distribution of trace metals such as Cu and Pb within natural and engineered nano-sized particles fraction. In the ternary system containing QDs, humic acids and metals, the amount of Cu or Pb bound to the QDs was 12 and 5 times higher than that associated to the SRFA or SRHA, respectively. Environmental implications of the results are discussed with respect to the role of nanoparticles as potential trace metal vectors and consequences on metal bioavailability was exemplified by measuring the bioaccumulation of Cu and Pb in tree green microalgae differing in their cell-wall composition.

Screening of forensic and clinical samples: from immunoassay tests to SWATH acquisition

Stefan König
Forensic Medicine, University of Bern, Switzerland

Fast and accurate screening of forensic and clinical samples is an important step for further decisions regarding the treatment of the patients or possible quantification of the detected compounds in body fluids. Up to date, immunoassays are still the most common techniques for screening, even if these biochemical tests exhibit some significant disadvantages such as cross reactivities or lack of sensitivity for specific compounds. Based on the latest generation of QqTof mass spectrometers – which are capable of acquiring complete MS and MS/MS spectra at a rate of 20 Hz and high mass accuracy – a new screening methodology was implemented in our typical workflow. Our methodology is based on Swath acquisition and high resolution/high accuracy reference spectra which are used for data processing. Several forensic cases will be presented and the impact of this mass spectrometry based technique in the field of forensic toxicology will be discussed.

Differentiation of dental bacteria in in vitro *cultures and human saliva by secondary electrospray ionization - mass spectrometry*

Lukas Bregy,[1] Annick R. Müggler,[2] Pablo Martinez-Lozano Sinues,[1] Diego Garcia Gomez,[1] Georgios N. Belibasakis,[3] Malcolm Kohler,[4] Patrick R. Schmidlin,[2] and Renato Zenobi[1]

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[4]Department of Pulmonology, University Hospital Zürich, Switzerland

Introduction

Periodontitis in humans is mainly caused by the four periodontal pathogens Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Tanerella forsythia and Treponema denticola. Therefore the fast and safe identification of the bacterial strains in saliva would be really useful. Secondary electrospray ionization – mass spectrometry (SESI-MS) is a technique that is suitable to solve this kind of analytical problem.

Methods

Our SESI-MS consists of a home-built ambient pressure ionization source equipped with a nano electrospray (water doped with 0.1 % formic acid) installed on a commercial high-resolution mass spectrometer (TripleTOF 5600+, AB Sciex). For detailed compound identification the SESI source was adapted for operation with a LTQ-Orbitrap mass spectrometer (Thermo Scientific). The headspace from bacterial cultures and humans (patients / healthy controls) was injected with a gas-tight syringe into the mass spectrometer without further sample preparation.

Preliminary Results

We were able to identify 34 compounds in in vitro bacteria samples, which are specific for A. ctinomycetemcomitans (n=8), P. gingivalis (n=18), T. forsythia (n=7) and T. denticola (n=1). In an exploratory part of this study, we measured the intensities of these 34 target compounds in the saliva of a periodontitis patient and two healthy controls. The patient was infected by P. gingivalis, T. forsythia and T. denticola. We were able to detect 5 compounds that are specific for T. forsythia and T. denticola with enhanced ion intensities in the saliva of the patient.

Novel Aspects

SESIS-MS has the potential for untargeted analysis of the metabolomic fingerprint of bacteria in in vitro cultures. In addition, the method can be used for rapid identification of the individual bacterial strains in biological in vivo samples like human saliva. In the future, the technique could help to diagnose such diseases without time-consuming microbiological tests.

Acknowledgement

This research was supported by a Swiss National Science Foundation (LB / 149617) and a Marie Curie European Reintegration Grant (PMLS) within the 7th European Community Framework Program (276860).

Capillary electrophoresis for on-site analysis

Israel Joel Koenka, Jorge Sáiz, Thanh Duc Mai, Peter C. Hauser

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Capillary electrophoresis (CE) is a straightforward separation technique for all ions which essentially only requires an inexpensive capillary and a high voltage supply. Quantification is possible by entirely electronic means in the form of capacitively coupled contactless conductivity detection (C4D). It is therefore feasible to construct relatively simple and inexpensive instruments. These can be battery powered and field portable.

Several iterations of such instruments have been designed in our laboratory. The most simple versions require manual operations for injection and capillary flushing, but these steps can also be automated. Sometimes anions and cations need to be determined in the same sample, a task which requires different operating conditions and therefore cannot usually be carried out at the same time. However, the simplicity of CE allows the construction of portable dual capillary instruments for concurrent determinations. Long term, unattended monitoring is a special challenge, which can also be addressed by CE.

A range of applications has been demonstrated. These include the analysis of waste water at a treatment plant, the determination of beta-agonists in pig feed, scopolamin in forensic samples, inorganic ions in pore water of lake sediments and consumer fireworks as well as nutrients on rock surfaces. Of particular relevance in some of these applications was the ability of CE to deal with very small sample volumes down to the low micro-litre range. A further benefit is the possibility of carrying out fast separations within less than a minute.

Ion-selective nanospheres as heterogeneous indicator reagents in complexometric titrations

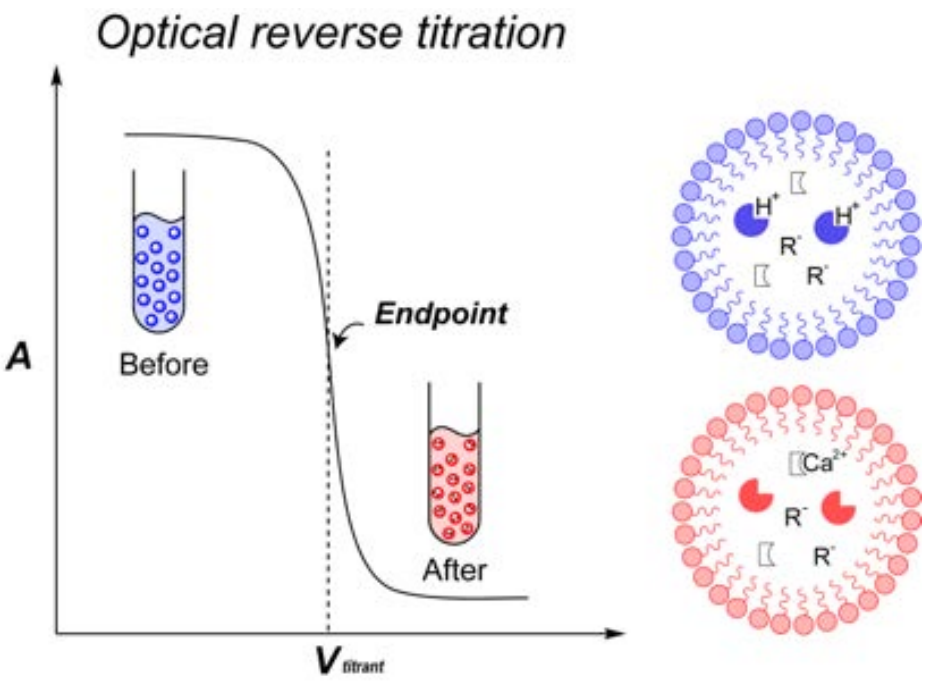
Jingying Zhai, Xiaojiang Xie, and Eric Bakker

Department of Inorganic and Analytical Chemistry, University of Geneva,
Quai Ernest-Ansermet 30, CH-1211 Geneva, Switzerland

Complexometric titration is a mature analytical technique that is being taught all over the world in analytical science. Titrations are routinely used to determine ion concentration, speciation as well as complexation reactions in various fields such as environmental, clinical and bioanalytical chemistry.

Traditionally, optical titrations of inorganic ions are based on a rapid and visible colour change at the endpoint with water soluble organic dyes as indicators. However, their pH dependent complexing ability and rather rigid selectivity remains an issue.

We present here alternative, heterogenous ionophore based ion selective nanospheres as indicators and chelators for optical titrations. The indicating nanospheres rely on a weaker extraction of the analyte of interest by ion-exchange, owing to the additional incorporation of a lipophilic pH indicator in the nanosphere core. Ca^{2+} titration was demonstrated as a proof-of-concept. Both the chelating and the indicating nanospheres showed good selectivity and a much wider working pH range than traditional complexometric approaches.



Reference

[1] J. Zhai, X. Xie and E. Bakker, Anal. Chem., **2015**, *87*, 2827–2831.

Poster Abstracts

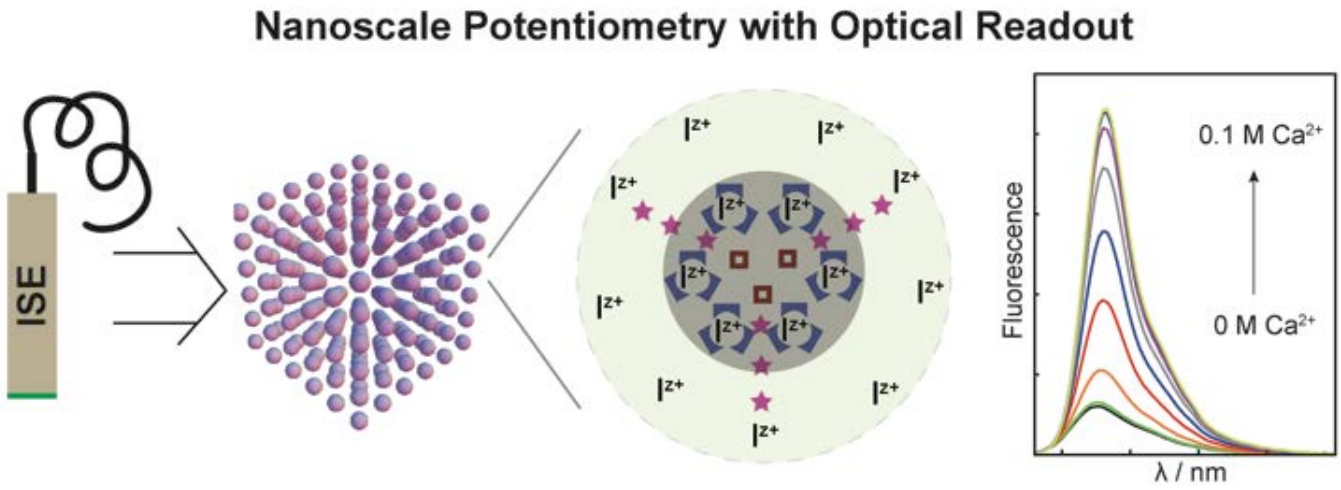
Potentiometric response from ion-selective nanospheres with voltage-sensitive dyes

Xiaojiang Xie, Jingying Zhai, and Eric Bakker

Department of Inorganic and Analytical Chemistry, University of Geneva,
Quai Ernest-Ansermet 30, CH-1211 Geneva, Switzerland

Ionophore-based ion-selective electrodes (ISEs) have been widely applied in clinical and environmental monitoring during the past few decades. ISEs require the implementation of conducting wires for signal transduction, which unfortunately, is impractical for the readout of individual nanoparticles.

It is here demonstrated for the first time that the potentiometric response of ion-selective nanospheres can be observed with voltage-sensitive dyes, thereby converting nanoscale electrochemical signals into an optical readout. No reference electrode is needed since the readout is by fluorescence. The results strongly support the potentiometric origin for the fluorescence response. The ion selective nanospheres exhibit excellent selectivity and respond to ion concentration changes independent of sample pH. K^+-sensitive nanospheres incorporating a lipophilic VSD were readily delivered into the social amoeba Dictyostelium discoideum in a non-invasive manner. This type of nanoscale optical ion sensors forms a promising new platform for intracellular ion quantification and imaging.



References

[1] Xiaojiang Xie, Jingying Zhai, and Eric Bakker, J. Am. Chem. Soc., **2014**, *136*, 16465–16468.

[2] Xiaojiang Xie, Agustín Gutiérrez, Valentin Trofimov, Istvan Szilagyi, Thierry Soldati, and Eric Bakker, Chimia, in press.

Absorbance detector based on a deep UV light emitting diode for a narrow column HPLC

Duy Anh Bui,[1,2] Benjamin Bomastyk,[1] Peter C. Hauser[1]

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The deep UV range (below 300 nm) is of great interest for the absorption detection with analytical instruments because the majority of organic species absorb in this region. The release of commercial deep UV light-emitting diodes (UV-LEDs) with the advantages of high efficiency and stability, long life time, and especially narrow emission bandwidth in recent years opens up the possibility to build powerful, simple, inexpensive and low power consumption devices by replacing conventional discharge lamps and monochromators or filters with UV-LEDs. An absorbance detector for narrow column HPLC based on 255 and 280 nm deep UV-LEDs and UV photodiodes was employed successfully for separations using an 1 mm ID column in isocratic as well as gradient elution. Three orders of magnitude linear response and detection limits down to the low micromolar range were achieved. This detector is useful for analytical applications in which only limited sample volumes are available or reduction of eluent consumption is desired.

Quantification of La in $CaMnO_3$ Perovskite by UV-fs-LA-ICPMS for Analysis of PLD Thin Films

Kevin Guex, Joachim Koch, and Detlef Günther

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The perovskite-structured compound $La_{1-x}Ca_xMnO_3$ exhibits interesting magnetoresistant properties, depending on what exact stoichiometry it has when formed into thin films via pulsed laser deposition (PLD) [1]. Laser ablation inductively coupled plasma mass spectrometry (LA-ICPMS) is one of the most promising techniques for bulk quantification of the raw material as well as lateral and depth profile analyses of any thin film derivatives thereof. However, LA-ICPMS applying a near infrared (NIR) femtosecond (fs) laser emitting at a wavelength of 800 nm in combination with single point calibration using NIST silicate glass reference materials proved challenging mainly due to differing LA thresholds and optical penetration depths. The very different LA thresholds prevented even simple bulk analysis of PLD thin films deposited on silicon wafers. It also turned out that even threshold-close fluences resulted in total removal of all material and copious amounts of substrate with the initial laser pulse. This has previously prevented any depth-profiling attempts.

Now, the usage of a novel UV-fs-LA system emitting at 257 nm and 206 nm allowed lowering the optical penetration depth, thus significantly decreasing material up-take rates per laser shot when operating at low fluences (0.22 J/cm^2). As a result, laser ablation of solely the PLD thin films became possible and made them accessible to surface scanning and depth profiling via UV-fs-LA-ICPMS. First results of both the analyses of perovskite thin films and scanning electron microscopy (SEM)-based morphology studies of craters formed by low-fluence UV-fs-LA will be presented and discussed.

References

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Quantification of 20 metabolites from erythrocytes infected by P. Falciparum by ion pairing liquid chromatography mass spectrometry (IP-LC-SRM/MS)

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Malaria, 15th worldwide disease is induced by a parasite called plasmodium. The infection by the P. falciparum form may result in severe symptoms, with high probability of death. During the blood stage, the parasite evolves and the last stage will conduce to the lyses of the erythrocytes.

Understanding the metabolomic modifications of infected red blood cells can provide insights how the pathways are affected by the presence of the parasite. For that purpose, 20 metabolites from multiple pathways (glucose, glucose 6P, fructose 6P, 3 phosphoglyceric acid, lactic acid, pyruvic acid, l-alanine, acetylCoA from central carbon metabolites, citric acid, succinic acid, fumaric acid, malic acid, L-aspartic acid, GABA, glutamic acid from tricarboxylic acid cycle, d-ribose 5P from pentose phosphate pathway and glycerol 3-phosphate, l-leucine, l-isoleucine, l-valine) were selected for quantitative analysis. These metabolites have highly hydrophilic and acidic properties affecting good retention on C_{18} stationary phases. To improve the chromatographic retention and separation, we have investigated hydrophilic interaction liquid chromatography (HILIC) and ion pair liquid chromatography (IP-LC). Zwitterionic columns (ZIC-HILIC) [1] have been shown to be better than amide columns according separation and retention criteria for these metabolites. HILIC columns have the advantage of low backpressure and more suitable in term of mobile phase for ion spray sources [2]. While the analytes are well retained, large peak width at base and tailing are observed for the metabolites of interest jeopardizing the quantification. IP-LC using hexylamine as ion pair agent [3] allowed to increase of the retention time and good peak shape for 17 on 20 metabolites (except: lactic, malic and acetylcoA) without significant loss in MS response. Finally the liquid chromatography method chosen is using ion pairing agent with a C_{18} column was applied to analyze the real samples.

Selected reaction monitoring acquisition in negative mode was used to quantify the 20 targeted metabolites. Glucose 6P, fructose 6-P as isoleucine and leucine are isomers and not well separated. Glucose 6P and fructose 6-P have a different fragment ion that is not the case for isoleucine and leucine. These last are quantified together.

The calibration curve is performed according an accuracy of ±20% and at least 2 orders of magnitude. The metabolite quantification is with 107 red blood cells infected by the parasite.

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Metabolite monitoring in fed batch cell cultures using MALDI TOF MS

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The analysis of intracellular metabolites is an important task to routinely monitor biotechnological fed batch process reproducibility and performance. Traditionally, these metabolite levels are followed using liquid chromatography (LC) combined with UV detection. However the long LC-UV runtimes compromise the possibilities to regulate a running process via feedback. We are presenting a novel MALDI-MS method that enormously reduces the time to analyze intracellular metabolites, and provides excellent robustness. A commercial MALDI TOF instrument (5800, ABSciex, Germany) and a microarray sample target for mass spectrometry were used in the study. The sample target consists of a coated and micro structured ITO glass slide. Hydrophilic spots on the otherwise omniphobic chip allow for fast, automated aliquoting and focusing of any organic solvent. Furthermore, a short extraction protocol was developed and used to monitor fed batch reactors. Adenosine-5´triphosphate was detected and quantified using an isotopically labeled internal standard ($^{13}C^{15}N$-Adenosine-5´-triphosphate). The appearance of ATP is in good agreement with literature data. The di- and monophosphates of adenosine as well as guanisine, cytidine, uridine were also monitored. Any methodologically induced analyte fragmentation or hydrolysis was corrected for. Moreover, the recorded metabolite profiles were analyzed using statistical tools, e.g. principal component analysis. The presented method has high-throughput capabilities and can potentially be applied in an industrial environment. The detected metabolite profiles were cross-validated using HPLC-UV. Development and implementation of a microarray for metabolite analysis of fed batch cultures using MALDI-MS. Fast and effective analyte extraction protocol combined with an analysis method including isotopically labeled internal standard.

A selective electrochemical online desalinator for in situ *environmental monitoring: from simulation to experiments*

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Our group recently introduced an online electrochemical desalination method that may be promising as a pretreatment step for a number of analytical methods that cannot be directly applied in injected samples of high salinity.[1] This module consists of the coaxial arrangement of an inner silver element inserted in a tube made of a cation-selective Donnan-exclusion membrane (such as Nafion), itself placed into an electrolyte containing a counter and reference electrode. An electrolysis protocol allows for the non-selective removal of cations and the selective removal of halides from the sample, leaving anionic nutrients in the sample for subsequent on-line analysis.

A number of parameters strongly influence the behavior of the desalination cell and include the dimensions of the cell, the applied voltage and the rate of fluidic control. Analyzing the effect of each parameter empirically is time and resource consuming, but mathematically, these parameters are all linked by a set of partial differential equations in space and time.

Since a precise solution is not possible, we developed a 2-dimensional finite-difference protocol to simulate the characteristics of the coulometric desalination device under different conditions. This allowed us to have a better understanding of the setup, thus improving both the injection and electrochemical conditions of the experiment.

We then assess the ability of the device to carry on reliable on-line potentiometric analysis of seawater samples.

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Target plate material influence on C_{60} LDI efficiency

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In order to establish the validity of models thus far proposed for matrix-assisted laser desorption/ionization (MALDI), such as disproportionation ionization,[1] the "lucky survivor" model,[2] cluster formation[3] and exciton pooling,[4] a basic LDI approach, based on electrospray deposition of C_{60} (stable, clear fragmentation pattern and ionizable in both negative and positive mode) on a wide range of target materials, with respect to their heat conductivity and electrical resistivity, was chosen as a starting point. The ion yield was monitored in both positive and negative polarity on a commercial MALDI-TOF MS instrument (Bruker Autoflex, Nitrogen laser, λ: 337 nm) varying both the laser fluence (0 – 3.53 J cm^{-2}, Δ 10%) and ion extraction delay time (0 – 950 ns, Δ 50 ns).

The presence of one or more maxima in the resulting ion yield plots for different extraction delay times could be an indication for the number of ionization mechanisms contributing to LDI and the time frames in which they operate. The differences in ion yield could possibly be attributed to the conductive properties of the substrate material upon which the sample was deposited.

For a statistically significant outcome the sample deposition needed to be rendered more reproducible. This was done by electrospraying C_{60} with an automated, custom-made setup with sliding Teflon® masks, preventing cross contamination. 10 x 10 spots with a 3 mm Ø were made on the metal insets to be tested, which were subsequently fitted into a milled-out MALDI sample target plate, whilst ensuring equal surface height. Per sample spot and condition, 3 x 250 spectra were averaged and processed in an automated fashion (MATLAB): integrating the spectra over a 360-725 m/z range, averaging the resulting areas under the curve for repeating experiments and finally generating a signal intensity profile accompanied by a standard deviation plot.

Results obtained thus far show that the C_{60} ion yield in positive mode is fourfold higher than its negative counterpart for all substrate materials tested, indicating that it is easier to remove an electron from C_{60} than to capture one during the plume formation process. Furthermore, the negative ion yield increases strongly for substrates with a low electrical resistivity and a low heat conductivity, which suggests that preventing charge leaking away from the area of impact increases the ion survival rate. All trends remain to be verified on alternative commercial MALDI-TOF MS instruments, but are a strong indication that substrate material choice should not be underestimated and that careful selection could prove beneficial to the ion yield, allowing for more sensitive (MA)LDI measurements.

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*Omics methods to find biomarkers of glucocorticoid exposure in zebrafish (*Danio rerio*) embryoss*

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Synthetic glucocorticoids (GCs) are frequently used in medicine. These compounds were detected in the aquatic environment in the ng/L range. GCs mimic cortisol, the natural stress hormone, by acting through the glucocorticoid receptor (GR) and altering target gene expression. Endogenously, cortisol is produced in fish when the hypothalamus-pituitary-interrenal (HPI) axis is activated. In our work, zebrafish embryos are used to investigate whether exposure to environmentally relevant concentrations of a model GC, clobetasol propionate (CP), results in GC-related effects, such as impairment of the stress response and immuno-suppression.

We avoid using an organic solvent carrier, such as DMSO, due to the experienced solvent effects. We are focusing on nanomolar concentrations, and since CP can be dissolved in the embryo medium up to 1000 nM, no precipitation is expected. Zebrafish embryos are exposed from 96-120 hpf for the omics analyses.

Targeted proteomics analyses are conducted on tryptic peptide digests of embryos exposed up to 100 nM CP. The proteotypic peptide targets are monitored using liquid chromatography coupled to mass-spectrometry (LC-MS) based targeted proteomics technique, selected reaction monitoring (SRM). GR target genes already examined in zebrafish at the mRNA level were selected to monitor their protein products. The targeted proteins have different physiological roles, e. g. myogenesis, vitamin D metabolism, immune function, and cardiogenesis. The SRM assays are being developed by using the selected proteotypic peptides synthetized in order to find the most intense transitions (i. e. m/z of the peptide and m/z of one of its fragment) to monitor in the embryo extracts.

Non-targeted metabolomics approach is being used to monitor the hydrophilic fraction of metabolites in zebrafish embryos after exposure up to 10 nM CP. The hydrophilic metabolites were analyzed using an LC-MS system. The runs were analyzed by Finding Molecular Features algorithm followed by multivariate data analysis (MVDA). Metabolites were also identified using a MS metabolite library of standards (MSMLS).

The described work characterizes the CP effects on the molecular levels. This study is focusing on finding potential GC-specific and sensitive biomarkers that can be used in developing a bioassay to monitor GCs in water samples. The bioassay will be used in effect-directed analysis (EDA) in order to identify unknown compounds able to interfere with the HPI axis.

All solid state carbonate selective electrode based on modified single walled carbon nanotubes as transducers

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Carbon dioxide is a well-known gas because of its contribution to global warming and its important role in metabolism and the pH regulation of blood. The determination of carbon dioxide both in the air or in solution is very important for clinical and environmental applications. As carbon dioxide can be dissolved in water and be transformed to bicarbonate or carbonate, and thereby forms an equilibrium between the three species. We may therefore get information about carbon dioxide and its speciation by measuring carbonate or bicarbonate instead of carbon dioxide.

Solid state electrodes have become sensitive and reliable tools for detecting ions in solutions for several decades, but its application for carbonate detection is still limited. Here, a new type of all solid state electrode using single walled carbon nanotubes (SWCNT) grafted with octadecylamine as inner transducers is introduced for the determination of carbonate in river samples.

Previously synthesized modified SWCNTs were drop cast on a planar glassy carbon electrode surface to form a homogeneous layer as transducer. A cocktail containing carbonate ionophore, tridodecylmethylammonium chloride(TDMACI), bis(2-ethylhexyl) adipate(DOA), polyvinyl chloride(PVC) and tetrahydrofuran(THF) was solvent cast on either the prepared transducer layer or a bare glassy carbon surface (control electrode), respectively. Potentiometric characterization of the electrodes with transducer shows improved analytical behavior than those without SWCNT in terms of carbonate selectivity, response slope, potential stability and repeatability.

The produced electrode was characterized for the determination of carbonate concentration in the Arve River and compared with the values obtained by standard volumetric titration, evaluating their usefulness for the real-time observation of carbonate variations in environmental systems.

High speed high resolution multi-elemental imaging of geological samples

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Elemental imaging has recently gained a lot of attention in the field of geology, biology and medicine, where the characterization of micro-structures and the evaluation of elemental distributions across heterogeneous samples are of major relevance.

Until now, most instrumental approaches applied in either two or three dimensional imaging studies were comprised of a laser ablation system coupled to a scanning (quadrupole or sector-field) mass analyzer. The fundamental operating principle of those systems did not allow simultaneous multi-element detection. In order to enable shot by shot detection, the system had to be operated at low laser repetition rates which made it suffer from spectral intensity skew errors and accounted for an extended data acquisition time. Laser spot sizes of a few 10 s of microns were commonly applied impairing high lateral resolution.

Here we report the coupling of an ArF excimer laser ablation system (λ = 193 nm) to a prototype ICP-TOF mass analyzer enabling high speed, high resolution, multi-elemental imaging of geological specimens.

Operating a fast-washout laser ablation tube cell, minimum signal widths of 9 ms were obtained when ablating NIST 610 (full width at 1% peak maximum). The system's capability to run at 100 Hz laser repetition rate while maintaining baseline separation of individual transient signals was demonstrated. Limits of detection in the sub µg/g range were observed for most trace elements. Full mass spectra can be acquired at a speed of up to 33 kHz.

Following the thorough characterization of the new setup performing different imaging experiments on standard reference materials, this LA-ICP-TOFMS setup was applied in quantitative two and three dimensional imaging studies dealing with various geological specimens including a cesium infiltrated Opalinus clay thin section with pyrite inclusions and a meteorite sample. Laser spot sizes varying from 1.5 to 5 µm and a laser repetition rate of 20 Hz were applied. The results of these studies will be compared to the findings made by synchrotron based micro-X-ray fluorescence spectroscopy. Advantages and shortcomings of both techniques will be discussed.

Direct voltammetric As(III) sensing in natural waters on a gel integrated renewable gold microelectrode

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Arsenic in the environment is of a global concern because of the widespread, chronic poisoning, found in a number of countries and affecting large populations. Inorganic As(III) and As(V) species are predominant in the water column, while organic forms are the main arsenic species in aquatic organisms. As(III) species are 60 times as toxic as the pentavalent salts and several hundred times as toxic as methylated arsenicals. The proportions of the inorganic species in the media are function of the bio-physicochemical conditions and thus may varies continuously in space and time [1]. Robust and sensitive analytical tools capable of direct, continuous in-situ As(III) sensing is therefore of prime interest for As health risk assessment.

For this purpose, we have developed a microelectrode consisting of a renewable gold nanoparticles plated Ir-based microelectrode (AuNPs-IrM) coated by a 350 to 650 µm thick agarose gel. This layer minimizes fouling problems by hindering diffusion of organic matters and inorganic colloids/macromolecules toward the sensor surface [2]. The Ir substrate pre-cleaning as well as the AuNPs deposition and renewal are fully controlled electrochemically. We report here on the Square Wave Anodic Stripping Voltammetry (SWASV) characterization of i) the flux mass of As(III) in the agarose gel as a function of the gel thickness and the temperature; and ii) the selectivity, sensitivity and long-term reliability of the sensor for direct As(III) measurements in synthetic and natural waters at pH 8. The results reveal that the gel-integrated Au-IrM fulfills the requirements for direct measurements in freshwaters [3].

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In-situ *study of trace metal dynamic and their synergistic interaction with phytoplankton communities*

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The bioavailable fraction of essential or toxic trace metals play an important role in phytoplankton growth, composition and metabolism. The concentration of bioavailable metal species may change substantially and continuously as a function of pH, T, water composition and biological activity. *In situ* monitoring of the bioavailable fraction of Cu(II), Pb(II), Cd(II) and Zn(II) can be performed using a gel integrated microsensor (GIME) based submersible voltammetric profiler (VIP) developed by Tercier-Waeber et al.

Our objective, as part of the Swiss National interdisciplinary project "Sensing Aquatic Microscale Heterogeneity", which started in 2014, is to apply the VIP under contrasting summer bloom conditions. The data bank obtained during several field campaigns will be used to study the dynamic of the bioavailable target analytes and their potential synergistic interaction with phytoplankton communities. For this purpose, the VIP has been coupled with a scanning flow-cytometer for microbial abundance and diversity analysis; sensors for macronutrients and multiparameter probes to perform simultaneous measurements at high spatial and temporal resolution in Greifensee surface waters.

We report here on the spatial and temporal data obtained from in situ simultaneous measurements of trace metals and master variables during a first field campaign in Lake Greifen along with a description of the set-up and future perspective.

Laser ablation ICPMS for single-cell-based tissue imaging

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Following immunohistochemical procedures, rare earth-tagged antibodies can be used as reporters to reveal rare cell subpopulations and tumor heterogeneity by mass cytometry, respectively. Whereas this has been previously applied to single cell analysis in cell suspensions [1], its application to tissue thin sections and combination with laser ablation (LA)-cy-TOF has gained a lot of attention [2]. Key features are high spatial resolution, fast image acquisition, high sensitive and multiplexed imaging capabilities under atmospheric conditions. The spatial resolution needed for a discrete sampling of tissues has to be in a range of 1 micrometer (or below) to provide insights into the anti-body distribution within single cells. Therefore, a low-volume "tube" ablation cell [3] was employed, which allows transport of LA-generated aerosols into the cy-TOF with minimum temporal dispersion to increase signal/noise ratios.

The research reported here followed up the progress recently made in the development of in-house built LA systems and low-volume ablation cells (geometries), which are suitable for a rapid screening of an entire tissue thin section. In this context, 213 nm and 193 nm laser wavelengths were evaluated for ablation. The use of an easier to operate solid state Nd:YAG-based 213 nm system was found to provide essentially identical characteristics with respect to spatial resolution and tissue ablation rates as the previously described Excimer-based 193 nm system.

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Ferrocene self-assembled monolayers as a solid contact ion-to-electron transducer for electrochemical sensors

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In conventional polymeric ion-selective electrodes (ISEs), the membrane is in a contact with the internal filling solution and the reference element. The replacement of these elements by solid contact ion-to-electron transducer avoids the counter-ion extraction at the inner membrane side.[1] However, the potential and electrochemical stability can be influenced by the leaching of redox-active species (e.g. oxidized form of ferrocene) from the membrane phase into the aqueous sample phase or the migration of charged species within the membrane to the buried interface once the potential/current is applied or by the formation of undesirable thin water layer at the buried interface of solid-contact ISEs.[2-4] In order to improve the stability of solid contact electrodes and develop more analytically robust sensors, several approaches have been proposed. The use of water-repellent copolymer (pMMA/pDMA) and the application of intermediate lipophilic layer, e.g. hydrophobic conductive polymers (POT) have been employed for this purpose.[5] More recently the application of redox-active self-assembled monolayers (SAMs) as an intermediate layer has been reported.[6]

Here, we present an approach based on SAMs on a gold substrate, which is built up in a two-step manner to provide a stable electrochemical sensor. In the first step, disulfide with terminated alkyne group is synthesized and covalently attached by self-assembly on a gold electrode and subsequently coupled with redox-active ferrocene azide or poly(vinyl chloride) chemically modified with azide groups using click chemistry. In the last step a thin polymeric ion-selective membrane was deposited on the modified substrate. The electrochemical behavior of the SAMs is characterized in detail with potentiometry, cyclic voltammetry and electrochemical impedance spectroscopy. Based on this work, we aim to develop all solid state ion sensors for clinical and environmental analysis.

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Sr isotope ratios and Rb-Sr ages by LA-ICPMS with isobar separation by on-line electrothermal vaporization

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High-precision Sr isotope ratio determination of solids by laser ablation multiple collector inductively coupled plasma mass spectrometry (LA-MCICPMS) is of big importance for a variety of applications. The accuracy of the $^{87}Sr/^{86}Sr$ determination is however limited by the isobaric interference of ^{87}Rb on ^{87}Sr. With the resolution offered by today's instrumentation, the two isotopes cannot be separated. Unlike in solution based methods, where the separation of Rb and Sr is possible [1], LA suffers from the fact, that all elements in the aerosol reach the plasma, leading to the mentioned interference. By heating the aerosol in an electrothermal vaporization (ETV) unit, changes in its chemical composition can be triggered. R. Brogioli used an HGA-600 ETV, which contains a graphite furnace for heating, to investigate the behaviour of heated laser-generated aerosols [2]. A partial suppression of the Rb-signal was observed, leading to improved $^{87}Sr/^{86}Sr$ accuracy. Selective Rb-evaporation and condensation at cooler furnace parts was suggested to lead to this effect, based on the different vaporization temperatures of Rb (< 1'000° C) and Sr (SrO ≈ 3'000° C). The goal of this work is to develop a standalone ETV unit to increase the Rb-suppression level and to improve the stability, as well as the reproducibility of the set-up. In a first step, the NIST 610 and BCR-2G standards were analysed by coupling either an ns-laser (213 nm) or fs-laser (257 nm) to the HGA-600. This set-up was compared with a prototype system based on a tungsten wire heater inline the aerosol stream. With the HGA-600 the Rb-signal suppression was found to start around 1'600° C, while the Sr-suppression occurred only at > 2'400° C. The new ETV prototype was also found to induce selective signal suppression for a range of elements as Ag, Cd In and Sn, whereas no separation between Rb and Sr occurred. This lack of efficiency is most likely due to the limited temperature that could be achieved with the prototype configuration. Additionally, gas leaks in the system caused W to evaporate at significant rate. As a next step, a modified system will be implemented, that is able to achieve higher wire temperature and avoids ambient air from reaching the heater to improve the separation efficiency.

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Ambient coupling of nano-liquid chromatography with dielectric barrier discharge ionization-mass spectrometryn

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Introduction

A plasma-based ambient ionization source, based on dielectric barrier discharge ionization (DBDI) and active sampling capillary, was coupled with a nano-liquid chromatographic instrumentation and employed in the ultra-trace analysis of diverse compounds. Identification of pesticides, amines and illicit drugs was performed by high-resolution and tandem mass spectrometry.

Methods

A complete vaporization of the eluent phase has to be achieved for an efficient coupling of a liquid chromatographic instrument with our DBDI source. A new vaporization system was built and used to vaporize the used flow of 800 nL/min. Incomplete vaporization leads to peak tailing and non-quantitative results. For pesticides detection, the instrumental method was tested with real matrices (apples and baby food), with QuEChERS extractions.

Results

Calibration curves were obtained for 16 pesticides with an LTQ-Orbitrap in fullscan mode (Resolution = 30000). Linear dynamic range at solution concentration was from 70-400 pg/mL to 1 µg/mL. Best limits of detection were 10 pg/mL, corresponding to an amount loaded on column as low as 50 fg and a gas phase concentration in the ppq range (as low as 70 ppq). Matrix effects did not produce a significant loss of analytes signal, confirming the robustness of the method. These results are currently being extended to a larger number of analytes, analyzed from complex matrices, including illicit drugs and different amines classes.

Conclusions

The coupling of a liquid chromatographic technique with our DBDI source is here presented. It opens new possibilities for the analysis of complex matrices, where the direct vaporization of samples containing multiple analytes is not always desired, due to, e.g., suppression effects. Different classes of analytes can be efficiently ionized and quantified in ultra-trace using mass spectrometry. Excellent linear dynamic range and linearity were obtained, with LODs way below the limit of legislation of 10 ng/mL for pesticides. This work represents one of the few applications of nLC to the analysis of environmental samples, the majority of which do not employ mass spectrometry for detection. The coupling of more widely used UPLC systems is in principle possible if an adequate vaporization of the liquid phase is achieved.