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Analytical Chemistry, Lecture **3**

Modern chemical ion sensor concepts based on electrochemically and optically triggered phase transfer

Eric Bakker, Ewa Grygolowicz-Pawlak, Xiaojiang Xie, Günter Mistlberger, Gaston Crespo, Majid Afshar, Bastien Néel and Alexey Shvarev

Highly selective ion complexing agents for the organic phase (ionophores) have been known for many years, but their main application in analytical chemistry has been in passive sensing devices, potentiometric and optical sensors.

This talk will introduce chemical concepts to trigger ion phase transfer reaction upon external stimuli, which result in a local perturbation of the sample phase that may give valuable complementary information about the sample. Electrochemical stimulation is shown to give rise to ion sensors that can exhibit a localized depletion at the membrane surface, which can be used to obtain information on total and so-called free concentrations at the same time. In a recent example, total calcium at physiological concentrations (2 mM) is shown to be quantitatively assessed in this manner without the need for altering the sample. In an extension of this work, thin layer coulometric ion-selective electrodes are being introduced, which allow one to selectively count out the ions from the thin layer sample, hopefully setting the stage for sensors that do not require calibration [1].

Optical ion sensors may also be stimulated with an external trigger to achieve a localized concentration perturbation of the sample. In one example, a fluorescent photoswitchable dye is found to exhibit a more than 6 pKa unit shift upon UV illumination [2]. This is used to trigger the extraction of ions from and to a polymeric phase, which has not only implications for the development of chemical sensors, but also the design of miniature devices that are capable of inducing a defined ion flux at a precise time and place.

[1] Grygolowicz-Pawlak, E.; Bakker, E. *Anal. Chem.* **2010**, *82*, 4537.
[2] Mistlberger, G.; Crespo, G.; Xie, X.; Bakker, E. *Chem. Commun.* **2012**, *48*, 5662.

Analytical Chemistry, Talk **4**

Revised EURACHEM/CITAC Guide on Measurement Uncertainty

Samuel Wunderli[1], Hanspeter Andres[1]

[1]Bundesamt für Metrologie METAS/Analytische Chemie, Lindenweg 50, 3003 Bern–Wabern, Switzerland

To ensure confidence in results from quantitative analytical chemical measurements traceability is of relevance. Metrological traceability is based on comparison of measurement results, that are a values and a corresponding uncertainties. The concepts in ISO-GUM [1] are recommended by international bodies such as IUPAC, IUPAC and others for all fields of measurements. EURACHEM/CITAC have issued now the third revision of their guide transferring the concepts to analytical chemical applications [2, 3]. In order to report a reliable uncertainty for a measurement value detailed knowledge of the nature of the measurand as well as the measurement itself is a prerequisite. The estimation of measurement uncertainty (MU) is not a mathematical task but rather demands critical thinking combined with the application of analytical knowledge. The new guide will be commented and the concept of measurement uncertainty including its terminology recently updated in VIM 3 [4] is explained. Different approaches to evaluate MU are outlined. Further the new sections of the revised guide are illuminated.

[1] Evaluation of measurement data – Guide to the expression of uncertainty in measurement, JCGM 100:2008, http://www.bipm.org/en/publications/guides/gum.html.
[2] EURACHEM/CITAC Guide CG 4, QUAM: 2000.1, Quantifying Uncertainty in Analytical Measurement, 2nd ed., http://www.eurachem.org/guides/pdf/QUAM2000-1.pdf.
[3] EURACHEM/CITAC Guide , QUAM: 2012.DIS1, Quantifying Uncertainty in Analytical Measurement, 3rd ed., http://eurachem.org/guides/pdf/QUAM2011_DIS1.pdf.
[4] International Vocabulary of Metrology – Basic and General Concepts and Associated Terms, JCGM 200:2012 (JCGM 200:2008 with minor corrections), http://www.bipm.org/en/publications/guides/vim.html.

Analytical Chemistry, Talk **5**

Nanoprobes for detecting DNA adducts

Ioannis A. Trantakis[1], Claudia Otto[1], Shana J. Sturla[1]

[1] ETH Zurich, Zurich, Switzerland

A selective colorimetric system for detecting O^6-alkyl-deoxyguanosine adducts is described. In preliminary studies we synthesized a new nucleoside analog (dPer) that forms a stable base pair with O^6-BnG. The objective of the present study was to exploit this binding phenomenon by incorporating this nucleoside analog into hybridization probes for a nanoparticle-based detection assay. Au nanoparticles, which are functionalized with alkanethiol-capped oligonucleotides containing modified nucleosides complex a 13-base oligonucleotide target. Hybridization of the target with the probes results in the aggregation of the nanoconjugates, which triggers a red to purple color change in solution. The stability of Au-oligonucleotide nanoconjugates was evaluated by measuring the temperature-dependent hybridization behavior of Au-oligonucleotide nanoconjugates containing Per paired opposite an O^6-BnG adduct in comparison with control sequences. The aggregates exhibit characteristic, exceptionally sharp absorbance transitions (monitored at 527 nm), which allow one to distinguish adduct containing target sequences from those containing canonical bases. Furthermore, when test solutions are incubated at different temperatures they have different colors according to the differential stability of the aggregates. Thus, O^6-BnG adducts can be detected visually without the need for any instrumentation. The nanoprobes we have developed and mechanistic information gained can lead, therefore, to the development of simple, low-cost and high-throughput detection of cancer-relevant DNA adducts.

Analytical Chemistry, Talk **6**

Performance Evaluation of Mass Spectrometry-Based Middle-Down Proteomics

Ünige A. Laskay[1], Luca Fornelli[1], Anton N. Kozhinov[1], Laure Menin[1], Karine Salamin[2], Michel Monod[2], Yury O. Tsybin[1]

[1]Ecole Polytechnique Fédérale de Lausanne, Lausanne, Switzerland
[2]Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland

A major problem in mass spectrometry (MS)-based bottom-up proteomics is the extremely high sample complexity generated by proteolysis, while top-down MS analysis is not yet applicable to a highly complex protein mixture. Middle-down proteomics (MDP) is considered a viable alternative by being fully compatible with the scan speed of the state-of-the-art FTMS instrumentation operating in LC-MS/MS mode. The main advantage of MDP is most noticeable when very high resolution (and mass accuracy) is applied to increase the protein identification confidence. Here, we advance MDP by characterization and application of novel endoproteases selective for adjacent basic residues within a polypeptide. The rationally-selected cleavage specificity results in peptides with a size of circa 5 kDa and, most importantly, an elevated charge state, facilitating high resolution FTMS and improving radical-driven MS/MS on chromatographic time scale.

In the MDP approach we seek to cleave the proteins into large, 5-15 kDa, peptides. Using in-silico digestion routine with an in-house Python-based bioinformatics tool we concluded that a protease that cleaves after two adjacent basic residues could be a candidate for true MDP. The sample complexity representing the number of peptides in MDP is therefore reduced approximately five-fold. Preliminary results indicate that employing the default fragmentation parameters HCD identifies the most peptides, followed by ETD and CID. To increase the sensitivity and further reduce the sample complexity, we employ in-spray supercharging using a two-channel nanoflow microchip. We will therefore present an array of innovations applicable to complex proteomic samples starting from sample preparation using novel enzymes, improvement in sensitivity by supercharging, and rapid detection at high resolution with advanced signal processing.

Analytical Chemistry, Talk **7**

Maximising resolution for UHPLC-TOF-MS metabolite profiling of complex natural samples – application to small and large molecules

Philippe J. Eugster[1], Daniel Biass[2], Davy Guillarme[1], Philippe Favreau[2], Reto Stöcklin[2], Jean-Luc Wolfender[1]

[1]School of Pharmaceutical Sciences, EPGL, University of Geneva, University of Lausanne, 30, Quai Ernest-Ansermet, 1211 Geneva 4, Switzerland
[2]Atheris Laboratories, case postale 314, 1233 Bernex-Geneva, Switzerland

In the LC-MS metabolite profiling of crude natural extracts, the development of methods providing high chromatographic resolution represents a key element. An efficient separation of coeluting LC peaks is indeed important for avoiding ion suppression and for obtaining high quality structural information on the highest possible number of the analytes present in a complex sample [1,2]. In the present study, some solutions for maximising the resolution of LC separations in a reasonable analysis time were experimentally established, based on a systematic investigation of the effect of different chromatographic parameters on peak capacity. The impact of the MS detector was also investigated, particularly the peak broadening due to the electrospray source. Two representative natural complex mixtures were used as models: a standardised extract of the medicinal plant *Hypericum perforatum*, containing small molecules ranging from 200 to 800 Da, and the venom of the predatory marine snail *Conus consors*, which contains numerous bioactive peptides with molecular masses ranging between 1'000 and 5'000 Da. The optimal generic profiling conditions applied to the *H. perforatum* extract and the *C. consors* venom provided peak capacity values higher than 700 and 1100, respectively. The application of these conditions in UHPLC-QTOF-MS/MS experiments provided exploitable MS/MS data for dereplication purposes.

[1] P.J. Eugster, D. Guillarme, S. Rudaz, J.L. Veuthey, P.A. Carrupt, J.L. Wolfender, *J. AOAC Int.* **2011**, *94*, 51.
[2] T. Koecher, R. Swart and K. Mechtler, *Anal. Chem.* **2011**, *83*, 2699.

Analytical Chemistry, Talk **8**

On-line Analysis of the coffee roasting process with PTR-ToF-MS: changes in flavor formation pathways for different coffee varieties

Alexia N. Gloess[1], Anita Vietri[2], Sandra Bongers[3], Thomas Koziorowski[3], Chahan Yeretzian[1]

[1]Zurich University of Applied Science, Institute of Chemistry and Biological Chemistry, 8820 Wädenswil, Switzerland
[2]Zurich University of Applied Science, Institute of Food and Beverage Innovation, 8820 Wädenswil, Switzerland
[3]Probat-Werke, 46446 Emmerich am Rhein, Germany

Chemical reactions during coffee roasting are leading to the formation of volatile organic compounds (VOCs) responsible for the aroma of a cup of coffee. Proton-transfer-reaction time-of-flight mass-spectrometry (PTR-ToF-MS) allows examining directly and in real-time the formation of these VOCs during the roasting process (Fig. 1) as a function of (i) the time-temperature-profile of the roasting process, and of (ii) the coffee variety being roasted [1]. This study, comprising eight different roasting profiles and five different coffee origins, revealed clear differences in the VOC formation between Robusta Malangsari, Arabica from Middle America and Arabica from Ethiopia. Minor differences are observable between Arabica from Columbia and Guatemala. Off-line analysis of the respective coffee brews revealed the differences in the cup, including sensory and instrumental analysis.

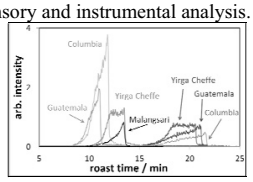


Fig. 1: Time-Intensity Profiles of $C_4H_4N_2$ (pyrazine) for different coffees and roasting profiles.

[1] Gloess, A.N.; Vietri,A.; Klopprogge, B.; Wieland, F.; Petrozzi, S.; Bongers, S.; Koziorowski, T. ; Yeretzian, C.; *in preparation.*

Analytical Chemistry, Talk **9**

Direct Access to Integral Membrane Proteins by MALDI-MS

Fan Chen[1], Kaspar Locher[2], Renato Zenobi[1]

[1]Department of Chemistry and Applied Biosciences, [2]Institute of Molecular Biology and Biophysics, ETH Zürich, Switzerland

A precise understanding of the chemistry of integral membrane proteins is important for optimizing crystallization conditions and for subsequent structure determination. Mass spectrometry (MS) is a powerful and versatile tool that provides deep insight into the state of integral membrane proteins. There are, however, only very few examples where electrospray ionization (ESI) - after quite a lot of efforts for optimizing buffer and instrument conditions - or matrix-assisted laser desorption/ionization (MALDI) have been successfully used for investigating integral membrane proteins or their complexes by MS. In all of these cases, the experimental conditions were highly specific and could not be easily transferred to other membrane proteins. In other words, it is still challenging for soft ionization MS to analyze integral membrane proteins and their complexes.

In this work, we succeded in the investigating integral membrane proteins in detergent micelles directly, using traditional dried droplet method, sinapinic acid as the matrix, and a commercial high-mass detector (HM2, CovalX) retrofitted to our MALDI-MS instrument. Oligosaccharyltransferase (PglB) was determined with a mass error around ± 0.1%. By protecting the membrane protein complexes via chemical cross-linking with glutaraldehyde, we unambiguously determined the subunit stoichiometry of a series of ATP-binding cassette (ABC) transporter complexes, the homomeric Campylobacter jejuni-encoded ABC transporter PglK, the heteromeric vitamin B12 importer BtuCD and BtuCDF. Moreover, the precise molecular weight generated allowed determining the site of N-linked glycosylation in Candida aibicans drug resistance protein 1 (Cdr1p). All the above information could not be obtained at all with SDS-PAGE or gel filtration chromatography, the commonly used tools in laboratories studying membrane proteins.

Analytical Chemistry, Talk **10**

Analysis of different phospholipid classes with LC-MS

Sutter Iryna[1], Arnold von Eckardstein[1], Thorsten Hornemann[1]

[1]Institute of Clinical Chemistry, University Hospital Zurich, Switzerland

Introduction

Phospholipids are ubiquitous compounds of heterogenious structure and functions. Physiological importance of phospholipids and their association with many diseases raises the interest in their detailed function. The understanding, how lipids function in biological systems helps to elucidate their pathogenetic contribution and their potential as biomarkers. Electrospray tandem mass spectrometry coupled with liquid chromatography is commonly used technique for characterisation of different phospholipid classes.

Methods

Using "soft" electrospray ionisation-mass spectrometry allowed us to analyse the different phospholipid molecular species. Application of the normal-phase liquid chromatography helped to avoid possible mass overlaps occurring during direct infusion analysis. Hence, liquid chromatography coupled with mass spectrometry is a powerful tool for assessing phospholipids, extracted from complex biological matrix. The developed LC-MS methods are capable to detect individual classes of phospholipids from total lipid extracts by either precursor ion or neutral loss scanning. This approach allowed characterisation of both the total endogenous amount of different phospholipid classes and of their individual molecular species.

Results

We developed an LC-MS method for specific detection of ceramides (CER), phosphatidylethanolamines (PE), phosphatidilglycerols (PG), phosphatidic acids (PA), phosphatidylcholines (PC), sphingomyelins (SM) and their lysoforms in plasma. All individual molecular species within these lipid classes can be detected in one analytical run using total lipid extract. The method allows semi-quantitative determination of different lipid classes.

Analytical Chemistry, Talk **11**

Filter diagonalization method-based mass spectrometry

Anton N. Kozhinov, Ünige A. Laskay, Konstantin O. Zhurov, Luca Fornelli, and Yury O. Tsybin

Ecole Polytechnique Fédérale de Lausanne, 1015 Lausanne, Switzerland

Fourier transform mass spectrometry (FTMS) provides a superior analytical performance among the palette of MS techniques for molecular structure analysis. However, to achieve the desired resolution of compounds in a mass spectrum, Fourier transform (FT) signal processing employed in FTMS instruments requires long data acquisition time compared to that needed for advancement of the FTMS-based applications. Recently developed filter diagonalization method (FDM) is capable of overcoming the FT resolution limitation and already demonstrated its performance in quantum mechanics calculations, NMR signal processing, and tracing modulation effects in FT-ICR signals. Here, we introduce FDM-based mass spectrometry (FDM MS) to accelerate data acquisition in FTMS [1,2]. Specifically, (i) we will present the FDM MS methodology, (ii) describe the custom-made software library for FDM-based signal processing and characterize its performance, and (iii) demonstrate FDM MS application for structural analysis of common classes of molecules and macromolecules, isolated and present in complex mixtures. In addition, we will discuss the current limitations of the FDM MS and technical aspects of its current implementation. The FDM MS performance is being characterized on a quad-core desktop computer, an eight-core workstation, and a multi-core computer cluster. The experiments were performed on a customized hybrid LTQ FT-ICR MS instrument (Thermo Scientific, Bremen, Germany) equipped with a 10 T superconducting magnet. The considered FDM MS applications are in bottom-up and top-down proteomics, metabolomics, and petroleomics.

[1] A. N. Kozhinov, Y. O. Tsybin, *Anal. Chem.* **2012**, *84*, 2850.
[2] S. M. Miladinović, A. N. Kozhinov, M. V. Gorshkov, Y. O. Tsybin, *Anal. Chem.* **2012**, DOI: 10.1021/ac2034584.

Analytical Chemistry, Talk **12**

Interfacing Droplet Microfluidics with MALDI-Mass Spectrometry

Simon K. Küster,[1] Stephan R. Fagerer,[1] Pascal E. Verboket,[1] Klaus Eyer,[1] Konstantins Jefimovs,[2] Renato Zenobi[1] and Petra S. Dittrich[1]

[1]Department of Chemistry and Applied Biosciences, ETH Zurich, 8093 Zurich, Switzerland
[2]Laboratory for Electronics/Metrology/Reliability, EMPA, 8600 Dübendorf, Switzerland

Here we present a novel method to interface a continuous-flow droplet-based microfluidic system with matrix-assisted laser desorption ionization mass spectrometry (MALDI-MS) by depositing droplets on high-density array plates at high throughput. The interface enables label-free detection and identification of molecules from individual droplets and combines the advantages of droplet microfluidics such as high throughput and ultra-low sample consumption with the high sensitivity (down to attomoles) and excellent mass resolution of MALDI-MS.

We created 3 nL microdroplets by injecting an aqueous phase into an immiscible oil phase (perfluorodecaline) using a microfluidic T-junction. The microchannel is connected to a capillary, the end of which deposits droplets on a standard-sized MALDI-MS plate mounted on an *x-y* stage. The plate is coated with a hydrophobic Teflon layer, which has been structured by laser ablation to form an array of 26444 hydrophilic spots of 300 μm diameter [1]. A simple optical detection system registers every droplet passing the capillary and automatically triggers the motion of the plate towards the next hydrophilic spot ensuring that only one droplet is deposited per spot. Reliable spotting frequencies of up to 8 Hz have been achieved. The hydrophobic coating of the plate prevents cross-contamination between adjacent hydrophilic spots and confines each droplet to a defined position within the array. As a first application an enzymatic reaction was successfully monitored.

[1] P.L. Urban, K. Jefimovs, A. Amantonico, S.R. Fagerer, T. Schmid, S. Mädler, J. Puigmarti-Luis, N. Goedecke, R. Zenobi, *Lab Chip* **2010**, *10*, 3206.

Analytical Chemistry, Talk **13**

Tracking isotopic signatures of CO_2 at Jungfraujoch with high-precision laser spectroscopy

P. Sturm, B. Tuzson*, S. Henne, L. Emmenegger

Empa, Swiss Federal Laboratories for Materials Science and Technology, Überlandstrasse 129, 8600 Dübendorf, Switzerland

A quantum cascade laser absorption spectrometer (QCLAS) is used for the first time to perform in-situ, continuous and high precision isotope ratio measurements of CO_2 in the free troposphere. The three main CO_2 isotopologue mixing ratios ($^{12}C^{16}O_2$, $^{13}C^{16}O_2$ and $^{12}C^{18}O^{16}O$) are simultaneously measured since December 2008 at the High Altitude Research Station Jungfraujoch (3580 m a.s.l.), Switzerland [1].

The high temporal resolution of the $\delta^{13}C$ time series allows the detection of pollution events and the application of the Keeling plot method for source signature identification. Backward Lagrangian particle dispersion simulations are used to determine the spatial origins of these CO_2 emission sources. In addition, our data reveal that $\delta^{18}O$, despite its complex nature, is a promising tool to assess the oxygen isotope exchange between atmospheric CO_2 and soil water. We suggest that the measured atmospheric $\delta^{18}O$ values are strongly influenced by the soil invasion process and, therefore, the apparent $\delta^{18}O$ source signatures may be used for experimental quantification of this effect at regional scales.

[1] Tuzson, B., Henne, S., Brunner, D., Steinbacher, M., Mohn, J., Buchmann, B. and Emmenegger, L., *Atmos. Chem. Phys.* **2011**, *11*, 1685–1696.

Analytical Chemistry, Talk **14**

Mass distribution of engineered metal and metal oxide nanoparticles measured by ICP-MS

Sabrina Gschwind, Daniel A. Frick, Joachim Koch, Detlef Günther

ETH Zurich, Wolfgang-Pauli Str.10, 8093 Zurich, Switzerland

Nanoparticles (NP), by definition smaller than 100 nm, have chemical and physical properties that are attractive for many different application fields. Nevertheless, there is little known about health and environmental related issues. Therefore, it is important to detect and characterize nanomaterial fast and sensitive regarding mass/size, morphology and elemental composition. One suitable technique for NP analysis is plasma spectrometry [1].

In 2011, the coupling of a microdroplet dispenser head, which allows introducing single droplets containing analyte and/or nanoparticles with a distinct temporal separation, to an Inductively Coupled Plasma Mass Spectrometer (ICP-MS) was achieved [2]. The setup was further revised for improved droplet transportation and to facilitate adaption to all commercial ICP-MS instruments.

With this technique it is possible to simultaneously access information about NP`s mass and composition. Therefore, two approaches for a mass calibration of NP were tested: Firstly, external calibration by measuring NP-free droplets doped with different concentrations of analyte solution and subsequent analysis of NP suspensions. Secondly, internal calibration by admixing analyte standard solution to NP suspensions and correlation of signal intensities arising from analyte-only-containing droplets to those induced by droplets containing both analyte solution and NPs. The results of these studies will be presented and discussed in detail.

[1] P.Krystek, A.Ulrich, C.C.Garcia, S.Manohar, R.Ritsema, *J. Anal. At. Spectrom.*, 2011, **26**, 1701-1721.

[2] S.Gschwind, L.Flamigni, J.Koch, O.Borovinskaya, S.Groh, K.Niemax, D.Günther, *J. Anal. At. Spectrom.,* 2011, **26**, 1166-1174.

Analytical Chemistry, Talk **15**

Combined ATR-IR/UV probe spectroscopy with hybrid hard-soft-modeling for solving the kinetic of metal oxide nanoparticles formation in benzyl alcohol

Idalia Bilecka[1], Sebastien Cap[2], Konrad Hungerbühler[2], Markus Niederberger[1]

[1]ETH Zürich, Department of Materials, Laboratory for Multifunctional Materials, Wolfgang-Pauli Strasse 10, 8093 Zurich, Switzerland. [2]ETH Zurich, Institute for Chemical and Bioengineering, Safety and Environmental Technology Group, Wolfgang-Pauli Strasse 10, 8093 Zurich, Switzerland

One challenge in the production of inorganic nanoparticles with specific properties is to control their formation *in-situ* and in real time. The complexity and the heterogeneity of the monitored species require a careful selection of the instrumentation as well as the use of powerful data analysis methodologies. In this contribution, combined spectroscopic monitoring, chemical kinetic modeling and simulation tools have been employed for the investigation of binary and ternary metal oxide formation. UV-Vis spectroscopy provided information about the solid phase (i.e. nanoparticle formation, band-gap evolution) while the liquid phase was monitored by infrared spectroscopy [1]. Chemometric resolution techniques, in particular the hybrid hard-soft multivariate resolution (HS-MCR) have been shown to be efficient methods for the rapid and accurate analysis of the complex infrared signal [2]. Combined spectroscopic signals and multivariate kinetic analysis gave access to a full kinetic description of divers metal oxide nanoparticle formation involving the precursor dissolution, the precursor-solvent reaction, the appearance and the evolution of the solid phase. Moreover, on the bases of kinetic simulations it has been shown that the kinetic parameters of individual precursor-solvent systems can be used for the prediction of the reactivity of mixed precursor systems.

[1] Bilecka I., et al., *ACS Nano*, **2009**, 3, 467.
[2] de Juan A. et al., *Chemometr. Intell. Lab. Syst*., **2000**, 54, 123.

Analytical Chemistry **16**

Evolution of the Green Fluorescence Protein upon transfer from solution to the gas phase.

Vladimir Frankevich, Konstantin Barylyuk, Paviel Sagulenko, and Renato Zenobi

Swiss Federal Institute of Technology, Wolfgang Pauli Str. 10, 8093 Zurich, Switzerland.

The combination of laser-induced fluorescence (LIF) with mass spectrometry opens up new possibilities for structural studies of biomolecular ions in the gas phase. What will happen to a protein when the solvent molecules are removed? Will it maintain a near-native conformation, unfold, or assume a new structure? The green fluorescent protein (GFP) is highly fluorescent in its native conditions, while it does not fluoresce upon unfolding. Fluorescence of GFP in the gas phase can therefore serve a very direct measure of its conformation.

We performed a series of experiments in order to probe the conformation of gas-phase GFP when trapped in the high vacuum of FTICR mass spectrometer. The trapped GFP ion population was subjected to CW laser irradiation of various power and exposure time. Interestingly, neither fluorescence nor dissociation was observed for trapped GFP ions. On the contrary, synthetic cR6G labeled protein-dye conjugates prepared from ubiquitin and GFP showed fluorescence signal when isolated in the FTICR cell under same conditions. The zero fluorescence signals could be directly attributed to protein unfolding in vacuum. On the other hand, the fluorescence could disappear due to the loss of several structural water molecules. Molecules of structural water build a hydrogen bond network that is necessary for GFP fluorescence. Our high resolution MS experiments show the absence of several water molecules in GFP. This water molecules loss does not affect on the GFP conformation too much but dramatically changes its fluorescence properties. Our preliminary data suggest that the latter mechanism is responsible for the lack of GFP fluorescence in vacuum.

Analytical Chemistry **17**

Oligonucleotide DNA duplexes as a model system to study relative electrospray response factors in monomer-dimer equilibria

Konstantin Barylyuk[1], Xueshu Xie[2], Renato Zenobi[1]

[1]ETH Zurich, Laboratory of Organic Chemistry, Wolfgang-Pauli-Strasse 10, 8093 Zurich, Switzerland
[2]Karolinska Institutet, Department of Medical Biochemistry and Biophysics, Scheeles väg 2, 17177 Stockholm, Sweden

Electrospray ionization mass spectrometry (ESI-MS) has emerged as a method for quantifying the binding affinity in non-covalent complexes. The relative peak intensities of binding partners and complex usually correlate with their abundances in solution. However, differences in ionization efficiency, ion transmission, and detection efficiency can disturb the peak ratios in the ESI mass spectrum and compromise the result of the binding affinity determination. Therefore, the relative electrospray response factor (R) is introduced in order to relate the ratio of the equilibrium concentrations to the experimentally measured intensity ratio.

Herein, we present a study of a model monomer-dimer equilibrium system to directly determine R experimentally. DNA oligonucleotides were chosen as a model system since in solution DNA exists as an equilibrium between the single strand (ssDNA) and the double helix (dsDNA). Moreover, the factors stabilizing dsDNA are very well understood, such that the Gibbs free energy of dimerization and the corresponding equilibrium constant (K_a) can be precisely determined experimentally or reliably predicted for a given nucleotide sequence and solution conditions. For a set of synthetic DNA oligonucleotides with the same chemical composition but different nucleotide sequence and therefore K_a, ESI-MS-based titration experiments were carried out in both positive and negative ion mode, and R values were extracted from non-linear curve fitting of the titration data. Our results suggest that ssDNA has a stronger ESI response and that the ssDNA-dsDNA ratio is distorted in positive ion mode, probably due to the disruption of the binding interface in dsDNA by protonation of the nucleobases.

Analytical Chemistry **18**

Calibration for High-Mass MALDI-MS: Concatenated Protein Oligomers as Calibration Standards

Simon Weidmann[1], Konstantin Barylyuk[1], Stefanie Mädler[1,2], Renato Zenobi[1]

[1]ETH Zürich, Department of Chemistry and Applied Biosciences, CH-8093 Zürich, Switzerland
[2]Current Address: Centre for Research in Mass Spectrometry, York University, Toronto, ON M3J 2R7, Canada

Mass spectrometry is one of the most widely used methods for the study of protein-DNA or protein-protein complexes. Compared to electrospray ionization (ESI), matrix-assisted laser desorption/ionization (MALDI) generates mostly singly charged ions. The signals of these ions give direct information about the masses of the investigated complexes. However, due to the low charge state the mass-to-charge ratio becomes rather high. Up to now, no suitable calibrants are available for accurate mass determination in the m/z range above 100 kDa.

We propose the use of concatenated protein oligomers with well-known masses as calibrants. An expression vector consisting of three maltodextrose binding protein (MBP) encoding genes was created. Specific recognition sites were introduced into the expression vector. These recognition sites allow for orthogonal proteolytic cleaveage of the concatemer. Furthermore an interaction site was introduced into the primary sequence, which allows for selective dimerization by formation of a disulfide bond. Since all calibration standards consist of several repetitions of the same protein the ionization efficiency is the same. Therefore mixtures of MBP oligomers with different masses can be used without ion suppression.

Using this system, several calibrants with different but well-known masses based on MBP subunits can be generated and a mass range from 40 up to approximately 500 kDa can be covered.

Analytical Chemistry **19**

Nanoscale Chemical Analysis by Tip-Enhanced Raman Spectroscopy: Recent Developments and Applications

Thomas Schmid, Lothar Opilik, Carolin Blum, Johannes Stadler, Renato Zenobi

Department of Chemistry and Applied Biosciences, ETH Zurich, 8093 Zurich, Switzerland

Tip-enhanced Raman spectroscopy (TERS) is an analytical technique that combines the chemical information provided by Raman spectroscopy with the signal enhancement at metallic nanostructures known from surface-enhanced Raman scattering (SERS) and the nanoscale spatial resolution of atomic force (AFM) or scanning tunneling microscopy (STM). Recent technological developments by our team include the optimization of tip fabrication by vapor coating of Ag onto AFM tips or by electrochemical etching of Ag wires to yield STM tips, the demonstration of AFM-TERS in a liquid environment [1], and the development of a novel illumination scheme for investigating opaque samples on metal substrates by STM-TERS [2]. The capability of STM-TERS for full-spectroscopic imaging of molecular thin films with high pixel numbers (e.g. 128 × 128 pixels), reasonable measurement times (down to 50 ms per pixel), and a lateral resolution of < 12 nm has been demonstrated. The technique has been applied for the determination of the distribution of two isomeric thiols in a mixed self-assembled monolayer [3], of defects, folds and contaminated areas in single-layer graphene [4] and of two lipids in a mixed supported lipid layer [5].

[1] T. Schmid, B.S. Yeo, G. Leong, J. Stadler, R. Zenobi, *J. Raman Spectrosc.* **2009**, *40*, 1392.
[2] J. Stadler, T. Schmid, R. Zenobi, *Nano Lett.* **2010**, *10*, 4514.
[3] J. Stadler, T. Schmid, L. Opilik, P. Kuhn, P. S. Dittrich, R. Zenobi, *Beilstein J. Nanotechnol.* **2011**, *2*, 509.
[4] J. Stadler, T. Schmid, R. Zenobi, *ACS Nano* **2011**, *5*, 8442.
[5] L. Opilik, T. Schmid, T. Bauer, J. Stadler, and R. Zenobi, *Phys. Chem. Chem. Phys.* **2012**, *13*, 9978.

Analytical Chemistry **20**

Amide I Mode Missing in Tip-enhanced Raman Spectroscopy?

C. Blum, T. Schmid, L. Opilik, N. Metanis, J. Stadler and R. Zenobi

Department of Chemistry and Applied Biosciences, ETH Zurich, Switzerland

Proteins are essential parts of organisms as they participate in nearly every cell process. Tip-enhanced Raman spectroscopy (TERS) is a surface sensitive analytical method that combines the advantages of two techniques: the high lateral resolution of scanning probe microscopy and the chemical information provided by Raman spectroscopy. Thus TERS has the potential to directly detect, e.g. proteins on a cell surface. In conventional Raman spectroscopy it is possible to investigate the secondary structure of the protein under investigation by looking at its amide modes. Especially the spectral position of the so-called amide I mode is sensitive to the secondary structure of the protein. By analyzing different proteins we want to approach the question if we can assign typical marker bands in their TER spectra. Additionally we want to investigate if and how TER spectra of proteins provide the information necessary to investigate their secondary structure.

We collected the conventional confocal bulk and TER spectra of several proteins and for comparison of phenylalanine (Phe) and Phe-Phe. The resulting spectra illustrate that the amide I mode is prominent in the confocal Raman spectra but not observable in the TER spectra. Additionally, we show the bulk Raman and TER spectra of two model heptapeptides – one with and one without an aromatic amino acid – to illustrate that the missing amide I mode is not due to an overwhelming dominance of the aromatic modes. The obtained results imply that it is possible to assign typical marker bands for proteins – as e.g. the aromatic modes – promising that imaging of proteins in a complex environment should be possible even though it seems to be unlikely that their secondary structure can be discriminated based on the amide I vibrational mode.

[1] C. Blum et al., Journal of Raman Spectroscopy, accepted (2012).

Analytical Chemistry **21**

Nanoscale imaging of 2D polymer films using tip-enhanced Raman spectroscopy

Lothar Opilik[1], Martin Spergl[1], Thomas Schmid[1], Thomas Bauer[2], Zhikun Zheng[2], Junji Sakamoto[2], A. Dieter Schlüter[2], and Renato Zenobi[1]

[1]Department of Chemistry and Applied Biosciences, ETH Zurich, 8093 Zurich
[2]Department of Materials, ETH Zurich, 8093 Zurich

Atomically thin, laterally extended two-dimensional films are of particular interest in material science because of their special properties that differ from any three-dimensional material. Methods to obtain two-dimensional films are scarce, and most approaches only result in very small fragments or lead to more extended but highly defective fims [1]. A very recent approach towards large one-atom thin films uses the formation of monolayered organometallic sheets from hexafunctional terpyridine monomers by complexation with metal ions (e.g. Fe^{2+}) at the air/water interface of a Langmuir-Blodgett trough [2]. These structures can be transferred to any kind of flat substrate where they are easily accessible to surface analytical methods. The present study deals with the characterization of these 2D polymeric structures by atomic force microscopy (AFM) combined with confocal Raman imaging. Additionally, tip-enhanced Raman spectroscopic (TERS) imaging was performed, yielding images with full spectroscopic information at every pixel and very high spatial resolution (< 12 nm was recently demonstrated on single-layer graphene [3]). The present work provides the opportunity to gain insight into the mechanism of 2D polymer formation and to identify defects down to the single molecule level.

[1] J. Sakamoto, J. van Heijst, O. Lukin, and A. D. Schlüter, *Angew. Chem. Int. Ed.* **2009**, *48*, 1030.
[2] T. Bauer, Z. Zheng, A. Renn, R. Enning, A. Stemmer, J. Sakamoto, and A. D. Schlüter, *Angew. Chem. Int. Ed.* **2011**, *50*, 7879.
[3] J. Stadler, T. Schmid, and R. Zenobi, *ACS Nano* **2011**, *5*, 8442.

Analytical Chemistry **22**

Real-time breath analysis by mass spectrometry

Christian Berchtold[1], Lukas Meier[1], Pablo Martinez[1], Youssef Daali[2], Marija Bosilskovska[2], Bernard Walder[2], Renato Zenobi[1]

[1] ETH Zürich Switzerland
[2]University Hospital Geneva, Geneva, Switzerland

Breath is a relatively simple matrix compared to blood or urine. Analyzed in real-time, breath could potentially provide direct information about a person's health status, medication and intoxication. To analyze breath, which contains various drugs and metabolites in low concentrations (ppbV to pptV) [1], a highly efficient analytical system with high sensitivity, selectivity, flexibility, real-time capability, and mobility is needed. Mass spectrometers equipped with an ambient ionization source potentially provide these capabilities. However, the sensitivity of available mass spectrometric tools is not yet sufficient to access compounds of high interest in breath, e.g., opiates that are investigated in one of our projects. Moreover such mass spectrometric tools need to be able to measure on-site, for example, in a hospital. Various new tools were developed and tested on site for our studies. A secondary electrospray ionization system was integrated into an artificial ventilation system, including a reference spray for validation [2]. An atmospheric pressure ion funnel was developed to enhance sensitivity [3], and mobile MS systems were tested in animal experiments. Volatile compounds like valproic acid [4] or nicotine were detected in the high ng/L concentration range in human breath, whereas low volatility components such as morphine could not be detected in pig's breath (detection limit: 0.5 ng/L). With the introduction of instrumental (e.g., ion funnel) and methodological improvements, we will soon be in a position to answer many metabolistic and pharmacokinetic questions by analyzing exhaled breath.

[1] F. M. Benoit et al, *Analytical Chemistry* **1983**, *55*, 805.
[2] C. Berchtold et al, *Int. Journal of Mass Spectrometry* **2010**, *2-3 145*
[3] L. Meier et al, *Analytical Chemistry* **2012,** *84, 2076*
[4] G. Gamez et al. *Chem. Commun.*, **2011**, *47, 4884*

Analytical Chemistry **23**

Examining breath profiles using secondary electrospray ionization mass spectrometry assisted by an ion funnel

Lukas Meier[1], Christian Berchtold[1], Stefan Schmid[1], Pablo Martinez-Lozano[1] and Renato Zenobi[1]

[1] Departement für Chemie und Angewandte Biowissenschaften, ETH Zürich, CH-8093 Zürich, Schweiz

It is a well established fact that analyzing breath allows one to draw conclusions about health or disease. While detecting the level of alcohol intoxication does not require highly sophisticated instruments any more, the detection of other medical conditions such chronic obstructive pulmonary disease (COPD) or even lung cancer turn out to be very difficult. In many cases, characterizing a patient's health status is based on signal patterns of unknown compounds. Developing more sensitive and highly diagnostic analytical techniques will in the future allow to assign theses signals, to make a direct connection to the body's metabolism.
In our experiments, we monitored more than 40 signals with m/z of up to 500 Th, extending the mass range usually used for breath analysis by more than 300 Th. In a control experiment without the ion funnel, considerably fewer peaks could be detected, thus not allowing breath profiling. By monitoring the breath of individuals over several weeks, we found their breath profile to remain very consistent over time. Inter-individual differences were found to be significant enough to easily identify single individuals based on their characteristic breath profile. Not surprisingly, food and drinks have a huge influence on the breath profile, usually "adding" a few peaks to, but not changing the breath profile substantially. Surprisingly, monitoring the breath profile before and after eating or drinking (chocolate, milk or coffee) showed that signals that originated from the food or drinks faded away within 10 minutes after consumption. However, during this period of time, signals that were previously seen within a volunteer's breath profile were greatly diminished in intensity, due to ion suppression.

Analytical Chemistry **24**

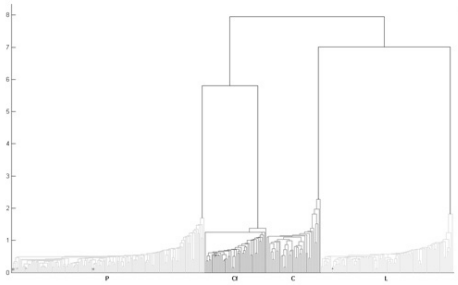
Monitoring intra- and inter-day human breath metabolic signatures: evidence for the existence of individual phenotypes

Pablo M-L Sinues[1], Lukas Meier[1], Christian Berchtold[1], Noriane Sievi[2], Giovanni Camen[2], Malcolm Kohler[2], Renato Zenobi[1]

[1]ETH Zurich, Department of Chemistry and Applied Biosciences, Switzerland
[2]University Hospital Zurich, Switzerland

A groundbreaking NMR study has suggested the existence of stable individual metabolic phenotypes in urine [1]. The analysis of breath is an attractive alternative approach to confirm the existence of such individual metabolic signatures because breath analysis is completely non-invasive.

Four subjects breathed through the counterflow inlet of a commercial mass spectrometer. The breath samples encountered an electrospray of water, whereby some exhaled compounds were ionized and readily detected. A total of 341 mass spectra collected during four days were analyzed statistically (see figure).



Dendrogram resulting from hierarchical cluster analysis of 341 breath samples donated by 4 subjects. In full agreement with [1], clustering according to donor is readily observable.

Our results support the existence of individual breath metabolic signatures.

[1] M. Assfalg *et al*, *PNAS* **2008**, *105(5)*, 1420.

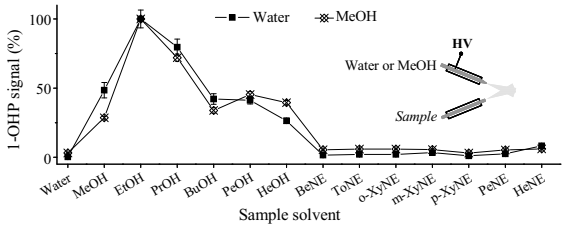
Analytical Chemistry **25**

Extractive Electrospray Ionization Mass Spectrometry of 1-Hydroxypyrene

Xue Li[1], Jiamo Fu[1], Renato Zenobi[2], Huanwen Chen*[3]

[1]Shanghai University, Shanghai 200444, P. R. China
[2]ETH Zürich, CH-8093 Zürich, Switzerland
[3]East China Institute of Technology, Nanchang 330013, P. R. China

High throughput mass spectrometric analysis of 1-hydroxypyrene (1-OHP) is highly desirable, especially for health risk assessment for people exposed to carcinogenic polycyclic aromatic hydrocarbons (PAHs) [1]. Extractive electrospray ionization mass spectrometry (EESI-MS) is a promising solution for this, and studies on EESI of 1-OHP are a prerequisite for the proper application of EESI-MS for detecting 1-OHP [2, 3].



Fourteen solvents of different polarities were used for delivering 1-OHP to an EESI source. When different primary ESI solvents (water and MeOH) were applied, the trends of 1-OHP signal variation with the sample solvent composition were almost the same. This work indicates 1-OHP ionization efficiency in EESI is also dependent on the sample solvent composition.

[1] P. Strickland, D.H. Kang, *Toxicol. Lett.* **1999**, 108, 191.
[2] X. Li, B. Hu, J. Ding, H. Chen, *Nat. Protoc.* **2011**, 6, 1010.
[3] W.S. Law, H.W. Chen, R. Balabin, C. Berchtold, L. Meier, R. Zenobi, *Analyst* **2010**, 135, 773.

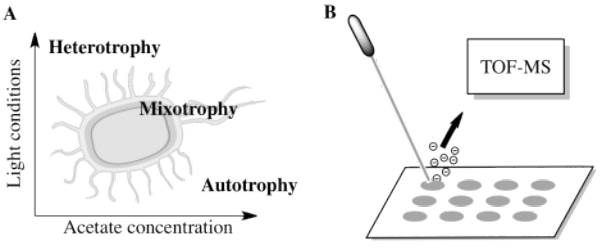
Analytical Chemistry **26**

Investigating algal food strategies in single cells using MALDI-TOF

Jasmin Krismer, Stephan Fagerer, Simone Nielsen, Alfredo Ibañez, Renato Zenobi

ETH Zurich/Department of Chemistry and Applied Biosciences, CH-8093 Zurich, Switzerland

Studying populations of unicellular organisms at the single cell level is essential for understanding heterogeneity in these biological systems. Even populations of genetically identical organisms display a substantial amount of phenotypical variability due to stochastic events. This variability renders the organisms capable of adapting to a changing environment and therefore contributes to their fitness [1].



We are investigating feeding strategies (heterotrophic, mixotrophic and autotrophic) in a genetically homogeneous population of *Chlamydomonas reinhardtii* caused by stochastic events providing different energy sources such as light and acetate (**A**). We use negative-mode matrix-assisted laser desorption/ionization (MALDI) mass spectrometry (**B**) to study metabolite profiles on single cell level in the alga [2]. Furthermore, we have investigated the liberation of cellular metabolites by biochemical and/or mechanical means in addition to the organic solvents.

[1] Acar M et. al. *Nat. Gen.* **2008**, *40*, 71-75.
[2] Heinemann M, Zenobi R, *Curr. Op. Biotech.* **2011**, *22*, 26-31.

Analytical Chemistry **27**

Evaluation of a selection of negative-mode matrices for metabolomics

Stephan R. Fagerer, Simone Nielsen, Alfredo J. Ibañéz and R. Zenobi

ETH Zürich, Wolfgang-Paulistr. 10, 8093 Zuŕich, Switzerland

Quantitative detection of a wide range of metabolites is a prerequisite to meaningful metabolomic studies. Matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) has been shown to be a useful method for this task due to its high sensitivity, with certain advantages over electrospray-based measurements, such as high tolerance for salts and contaminants, and a generally straightforward sample preparation [1].
Matrices that are suitable for metabolomics studies must fulfill several requirements, such as low chemical background in the mass region of interest (usually 50– 1500 m/z), high ionization efficiency for target compounds, and good co-crystallization (for a series of solvents). Only few matrices exist that meet all of these criteria. Examples of matrices that have been employed for the detection of small molecules in negative mode are: 9-aminoacridine (9-AA) [1], dihydroxybenzoic acid (DHB) [2], and 1,8-bis(dimethylamino)naphthalene (DMAN) [3]. In this study, we compare a variety of aminoquinolines and acridines for their ability to ionize amino acids, nucleotide phosphates, sugar phosphates and Krebs cycle intermediates. Aminoquinolines were found to be suitable for amino acid analysis, whereas acridines were favored for nucleotide phosphates. The influence of different substituents will be discussed, too.

[1] Vaidyanathan, S. and R. Goodacre, Rapid Commun. Mass Sp., **2007**, *21*, 2072.
[2] Cohen, L.H. and A.I. Gusev, Anal. Bioanal. Chem., **2002**, *373*, 571.
[3] Shroff, R., et al., P. Natl. Acad. Sci. USA, **2009**. *106*, 10092.

Analytical Chemistry **28**

Single-cells analysis by mass spectrometry: an insight into cellular heterogeneity

Robert F. Steinhoff[1], Alfredo J. Ibáñez[1], Renato Zenobi[1]

[1] Eidgenössische Technische Hochschule Zürich, Department of Chemistry and Applied Biosciences, CH-8093Zürich, Switzerland

Phenotypic heterogeneity is a widespread phenomenon that is correlated to fundamental fitness and development of organisms. [1] For example, the responses to antimicrobial treatment or other stresses can manifest itself in heterogeneity in terms of resistances, which finally leads to survival of a subpopulation. [2] Antimicrobial and insulin resistance in cell populations are two further examples that currently cannot be explained from population level measurements, and thus call for new, more sensitive and highly accurate analytical methodologies. Single-cell analysis is a rapidly developing field, which starts to provide insight into cell-to-cell variations like phenotypic heterogeneity and stochasticity-induced heterogeneity. [3]

Our approach to single-cell analysis combines a selective single cell isolation step on a micro array with subsequent mass spectrometric investigation of the lysed cell. Currently, the ionization technique in use (matrix-assisted laser desorption, MALDI; matrix: 9-aminoacridine) allows the identification of various metabolites (e.g., ATP and other nucleotides) of the central metabolic pathway in single cells. [4] We are focusing on the further development of our lab-made microarray for mass spectrometry (MAMS) technology for metabolite analyses, (in particular: the single cell spotting technique and the ionization techniques for mass spectrometry) to further push the limits of detections of relevant metabolites. We plan to demonstrate the heterogeneous cellular response of monocytic THP-1 cells towards external stress (e.g. calorie restriction or by addition of fatty acids).

[1] Heinemann, M. and R. Zenobi, Curr Opin Biotechnol. **2011**, 22, 26
[2] Sumner, E.R. and S.V. Avery, Microbiology **2002**, 148, 345
[3] Fritzsch, F.S., et al., Annu Rev Chem Biomol Eng. **2012**, online
[4] Urban, P.L., et al., Mol Biosyst. **2011**. 7, 2837

Analytical Chemistry **29**

Determination of protein-ligand binding constants of a cooperatively regulated tetrameric enzyme using electrospray mass spectrometry

Dragana Cubrilovic[1], Wolfgang Haap[2], Marcel Gubler[2], Armin Ruf[2], Renato Zenobi[1]

[1]ETH Zürich, Department of Chemistry and Applied Biosciences, 8093 Zürich
[2]F. Hoffmann-La Roche Ltd, Discovery Research, 4070 Basel

In this study we present an approach to investigate the allosteric mechanism in the binding of ligands to a multimeric enzyme using electrospray ionization mass spectrometry (ESI-MS). The advantage of ESI-MS compared to other methods is a direct visualization of the ligation states that are formed in equilibrium in solution. The model system chosen for this purpose is the homotetrameric enzyme fructose-1,6-bisphosphatase (FBPase), a potential therapeutic target for glucose control in Type-2 diabetes. Furthermore, FBPase can be implicated in glucose sensing and in regulating insulin secretion in β-cells. A series of inhibitors against FBPase was investigated to determine the dissociation constants (K_D) by ESI-MS. These inhibitors occupy the allosteric AMP binding site, which is the most easily druggable.
For quantitative determination of binding constants the Hill equation was extended for our particular model system. The K_Ds determined by ESI-MS correlate well with IC_{50} values measured in solution. In addition, we used our mathematical model system to estimate the Hill coefficient, which describes the number of inhibitor molecules required to bind to the FBPase. The ESI mass spectra allow one to deduce the cooperativity of the multimeric model system, that proves to be positive in case of the investigated model system.
This study highlights the benefits of ESI-MS as a fast and label-free method not only for determination of dissociation constants of cooperatively regulated enzyme, but also to better understand the mechanism of enzymatic cooperativity.

Analytical Chemistry **30**

Active capillary plasma source for ambient mass spectrometry

Maryia Nudnova[1], Liang Zhu[1], Renato Zenobi[1]

[1]ETH Zurich, Wolfgang-Pauli-str. 10, 8093 Zurich, CH

Mass spectrometry can potentially be used as an analytical technique for acquiring high localized chemical information. It has been shown that scanning probe microscopy combined with MS allows acquiring both μm-scale topographic and chemical imaging at ambient pressure. Higher spatial resolution means a smaller diameter of the ablation spot and consequently, smaller craters from which ablation takes place. Thus, efficient sampling and subsequent ionization of ablated material are of key importance for effective nanoscale chemical imaging at atmospheric pressure. Plasma based ambient ionization sources can provide both efficient sampling and ionization. In this work, we developed a plasma-based active capillary, which was optimized for performing surface analysis of solid samples with high spatial resolution. We constructed two types of active capillaries for ambient MS. One we refer to as the 'wire electrode' configuration, and the other as the 'cap electrode' configuration. Both types of active capillaries are suitable for analysis of vaporized liquids or solids. Sensitivity tests using liquid decylamine dissolved in water were made to characterize the different active capillary constructions. The lowest concentration of decylamine that could be detected by the 'wire electrode' system in the current experiments (i.e. sensitivity of the active capillary) was 0.1 ppb. The minimal concentration of decylamine, which was detected by the 'cap electrode' system, was 1 ppb. The “taper cap-tape” ion source is approximately ten times more sensitive that the “wire-tape” plasma ionization source. We demonstrated that the geometry of the electrodes plays a crucial role in terms of plasma parameters and ionization efficiency. A concentration of 0.1 ppb corresponds to 100 fmol of substance that is transported through the sampling capillary in 1 second. To demonstrate that the active capillary can be used for ionizing solid-phase analytes we carried out a series of experiments on a solid caffeine thin film and for anthracene crystals.

Analytical Chemistry **31**

The Capillary Gap Sampler: Development of a New Device for High-Throughput Analysis of Low Sample Amounts by ESI-MS

Volker Neu[1], Roger Steiner[2], Christof Fattinger[2] and Renato Zenobi[1]

[1] Department of Chemistry and Applied Biosciences, ETH Zurich, CH-8093 Zürich, Switzerland. [2] F. Hoffmann-La Roche AG, pRED, Pharma Research & Early Development, Discovery Technologies, Grenzacherstrasse 124, CH-4070 Basel, Switzerland

High-throughput analysis of compound libraries is a key issue in pharmaceutical drug screening. In terms of cost and resource control, the sample consumption plays an important role, thus miniaturized screening systems are preferred. Other requirements include a minimum dead-volume during sampling, the prevention of sample carry-over effects, as well as a detection method that provides high analytical information content. In this context, the so called capillary gap sampler has been developed, which can be directly coupled to electrospray ionization mass spectrometry (ESI-MS).
The sampler consists of two oppositely arranged capillaries with dimensions in the micrometer range, which are not tightly connected, such that a capillary gap of several nanoliters forms. One of the capillaries is used as a buffer flow line and the other one works directly as ESI spray needle. By applying a buffer flow, a liquid bridge is formed into which sample volumes of few nanoliters can be infused by a pin carrying a hanging droplet. The pin is moved by a fast micro robot for sample uptake and delivery. In order to control the flow dynamics, the liquid gap is enclosed by a pressure chamber, which allows a precise liquid bridge control *via* a dynamic chamber pressure and buffer flow regulation system.
Results show that sample delivery into the gap without touching any additional surface is feasible, thus minimizing carry-over effects. The systems also offers a quite small total dead-volume (< 150 nL), resulting in sample delay times and peak widths of less than 7 seconds. Moreover, direct monitoring of drug screening tests was performed, which underlines the system robustness in terms of spraying time without capillary clogging.

Analytical Chemistry **32**

Method transfer from HPLC to Middle Pressure Liquid Chromatography (MPLC) for efficient large scale isolation of natural products.

Challal S.[1], Queiroz E.F.[1], Guillarme D.[1], Wolfender JL.[1]

[1]School of Pharmaceutical Sciences, EPGL, University of Geneva, University of Lausanne, Quai Ansermet 30, 1211 Geneva, Switzerland

In natural product (NP) research, bioactive compounds are generally identified in complex natural extracts by bioactivity-guided fractionation. Such an approach is rather time-consuming and usually requires multiple chromatographic steps. For performing bioactivity screening on a large set of targets, mg amounts of NPs are needed. In order to obtain in one step mg amounts of pure NPs directly from crude plant extracts (tens of grams), a generic gradient transfer method that provide efficient separation up-scale from analytical HPLC to large preparative MPLC has been investigated. The proposed method takes the advantage of HPLC modeling based on generic linear gradients at the analytical level to maximize the separation of compound of interest in an extract. This step was performed with an HPLC column (250 x 4.6 mm i.d.) packed with the same C18 material than MPLC. The gradient was geometrically transferred to the MPLC level (920 x 49 mm i.d. column) by system characterization and chromatographic calculations. For a monitoring of the largest possible number of constituents, UV and ELSD detections were used at the analytical and preparative scale. MS monitoring was performed by post-chromatographic high throughput UHPLC-TOF-MS profiling of the aliquot of all fractions in the 96 well microtiter plate format. A rapid evaluation of the performance of the preparative scale separation was obtained after combination of all LC-MS profiles. Examples of one-step separation of crude plant extracts are presented and the possibilities and limitations of the approach discussed.

[1] Guillarme D, Nguyen, DTT, Rudaz, S., Veuthey, J.-L. *Eur. J. Pharm. Biopharm*. **2008**, 68, 430.
[2] Wolfender JL, Eugster, P, Bohni, N, Cuendet, M. *Chimia* **2011**, 65, 400.

Analytical Chemistry **33**

Ion mobility spectrometry applied to complex plant extract profiling: possibilities, limitations and outlook

Philippe J. Eugster[1], Richard Knochenmuss[2] and Jean-Luc Wolfender[1]

[1]School of Pharmaceutical Sciences, EPGL, University of Geneva, University of Lausanne, 30, Quai Ernest-Ansermet, 1211 Geneva 4, Switzerland
[2]TOFWERK AG, Feuerwerkerstrasse 39, 3602 Thun, Switzerland

Plant extracts are composed of hundreds of compounds, which vary widely in structure, physicochemical properties and concentration. Very high performance separation and detection are required to obtain detailed information on the composition of such complex extracts in the frame of metabolite profiling studies. LC-MS systems match these requirements and are thus widely used for metabolite profiling, especially due to recent advances (sub-2 µm LC particles, high-resolution MS) [1]. There is however an on-going need for better resolution in both LC and MS dimensions to improve the number of features detected. Coelutions and unresolved isomers remain frequent in plant extracts profiling. Ion mobility spectrometry (IMS) represents an additional dimension, based on the separation of the ions in the gas phase according to their chemical and physical interactions with the drift gas [2,3]. Coupled with a TOF-MS, IMS offers a high speed (milliseconds) separation dimension capable of resolving many of the isomers and stereoisomers not easily separated by LC. In this work, the potential of IMS for the deconvolution of isomeric flavonoids has been studied, and the capacities and complementarities of IMS-TOF and UHPLC-TOF-MS platforms for the metabolite profiling of the medicinal plant *Ginkgo biloba* have been compared.

[1] P.J. Eugster, D. Guillarme, S. Rudaz, J.L. Veuthey, P.A. Carrupt, J.L. Wolfender, *J. AOAC Int.* **2011**, *94*, 51.
[2] K. Kaplan, S. Graf, C. Tanner, M. Gonin, K. Fuhrer, R. Knochenmuss, P. Dwivedi, H.H. Hill, Jr., *Anal. Chem.* **2010**, *82*, 9336.
[3] P. Dwivedi, A.J. Schultz, H.H. Hill, Jr., *Int. J. Mass Spectrom.* **2010**, *298*, 78.

Analytical Chemistry **34**

Effect of nitrogen supply during grape-growing on wine composition unraveled by a metabolomic approach.

Guillaume Marti[1], Vivian Zufferey[2], Katia Gindro[2], Olivier Viret[2] and Jean-Luc Wolfender[1]

[1]School of Pharmaceutical Sciences, University of Geneva, University of Lausanne, CH-1211 Geneva 4, Switzerland
[2]Swiss Federal Research Station Agroscope Changins-Wädenswil, Route de Duillier, P.O. Box 1012, CH-1260 Nyon, Switzerland.

In recent years, terroir characteristics have become increasingly important for the wine industry. However, to improve the technical aspects of wine production, a better understanding of how environmental conditions affect fruit and final wine quality is needed. In addition to climate, soil also marks a major influence to the terroir effect but little is known about its main contributing factors. A recent study conducted in the Vaud viticultural area has shown that vine nitrogen content appeared to be one of the most important parameter that influence the vine-fruit-wine continuum [1]. Wines made from grapes with low nitrogen content had negative sensory characteristics and low aromatic complexity. In order to confirm these results, a large scale study on several sites during five seasons (2006-2010) in the Vaud vineyards has been undertaken on soil presenting the same characteristics. A given concentration of assimilable nitrogen has been supplied on leaves during the grape-growing to simulate the vine nitrogen content. To unravel the subtle biochemical changes induced by nitrogen supply on wine composition, a combined metabolomic approach based on reverse phase and hydrophilic interaction liquid chromatography TOF-MS and proton NMR fingerprints has been undertaken [2]. Several biomarkers in close relation to nitrogen supply could be highlighted by supervised data mining and identified by means of their accurate mass, fragmentation pattern and proton NMR.

[1] Jean-Sébastien Reynard *et al. J. Int. Sci. Vigne Vin*. **2011**, *45*, 211-221.
[2] G. Glauser, *et al. J. Chromatogr. A*. **2008**, 1180, 90-98.

Analytical Chemistry **35**

Miniaturization of fungal co-culture for the screening of new bioactive induced metabolites by MS-based metabolomics

Samuel Bertrand[1], Antonio Azzollini[1], Olivier Schumpp[2], Nadine Bohni[1], Michel Monod[3], Katia Gindro[2], Jean-Luc Wolfender[1]

[1]School of Pharmaceutical Sciences, EPGL, University of Geneva, University of Lausanne, quai Ernest-Ansermet 30, CH-1211 Geneva, Switzerland

[2]Mycology group, Agroscope Changins ACW, Route de Duillier, CH-1260 Nyon, Switzerland

[3]Departement of Dermatology and Venereology, Laboratory of Mycology, CHUV, CH-1011 Lausanne, Switzerland

In natural product field, the search of new sources of compounds is a key element. In this respect microorganisms have provided a large number of biologically active molecules [1]. Recently, the use of fungal co-culture for the induction of new compounds has emerged as a new field in drug discovery [2]. In this type of study a key step is the localization of induced metabolites in the confrontation zone of fungi co-cultures at the petri dish level by high throughput LC-MS based metabolomics. Another important aspect is the reproducibility of induction according to the co-culture conditions.

We present here the optimization of a miniaturized 12-well plates petri dish strategy used for LC-MS metabolite profiling of the fungal extracts. The method provided a satisfactory reproducibility and was used for the high throughput screening of induced biomarkers by a UHPLC-TOF-MS based metabolomic approach on a large panel of fungi. This study demonstrates the consistent *de novo* induction of new metabolites in co-culture conditions.

Acknowledgements: This work was supported by Swiss National Science Foundation Sinergia Grant CRSII3_127187 (to J.-L. W., K. G. and M. M.)

[1] Berdy J, *J. Antibiot.* **2005**, *58*, 1.
[2] Glauser G *et al.*, *J. Agr. Food. Chem.,* **2009**, *57*, 1127.

Analytical Chemistry **36**

Classification of lemon essential oils and selection of biomarkers by a multiblock analysis of UHPLC-TOF-MS, NMR and MIR data using multiple factor analysis

Florence Mehl[1], Guillaume Marti[1], Julien Boccard[1], Laurence Marcourt[1], Benjamin Debrus[1], Philippe Merle[2], Estelle Delort[2], Lucie Baroux[2], Horst Sommer[2], Jean-LucWolfender[1], Serge Rudaz[1]

[1] School of Pharmaceutical Sciences, EPGL, University of Geneva, University of Lausanne, 30, Quai Ernest-Ansermet, 1211 Geneva 4
[2]Firmenich, Corporate Research, Geneva, Switzerland

Differences in climate, soil or method of extraction confer specific organoleptic characteristics to lemon essential oil (EO). To authenticate and control the quality of essential oils it is mandatory to develop chemometrics and analytical tools that help classification issues. Qualified samples from Argentina and Italy, as main producers of lemon EO, were analyzed by UHPLC-MS, NMR and FT-MIR. The data obtained were processed [1] by a multiple factor analysis (MFA) [2].

MFA showed that each of the three analytical technique was consistent and convergent for classification. Furthermore, in the consensus model, where the three data sets are merged, investigation of biomarkers of interest is facilitated by the simultaneous interpretation of orthogonal spectroscopic informations (e.g. functional groups in NMR, MIR; distinct value for each detected analyte in UHPLC-MS). Our study demonstrates the usefulness of MFA for both classification of lemon EO and determination of related biomarkers.

[1] Bertrand, D. and Cordella, C. *www.chimiometrie.fr* **2011**
[2] Escofier, B. and Pagès, J. *Comput. Stat. Data. An.* **1990**, *18*, 121–140.

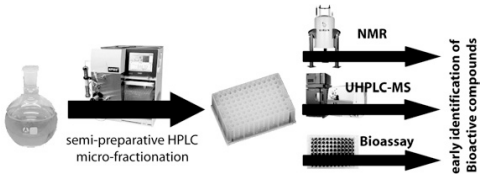
Analytical Chemistry **37**

Integrative approach in natural product research: at-line coupling of micro-fractionation with NMR, LC-MS and bioassays

Antonio Azzollini, Samuel Bertrand, Andreas Nievergelt, Julien Boccard, Serge Rudaz, Muriel Cuendet, Jean-Luc Wolfender

School of Pharmaceutical Sciences, EPGL, University of Geneva, University of Lausanne, 30 quai Ernest-Ansermet, CH-1211 Geneva 4, Switzerland

Recent approaches in natural product research are leading toward the discovery of bioactive chemical entities at the microgram level. In comparison to classical bioassay-guided fractionation, the use of LC-MS metabolite profiling in combination with micro-fractionation for both bioactivity profiling and NMR analysis, allow the identification of bioactive compounds at a very early stage [1].



As an example of such integrative micro-fractionation approach, the extract of *Tofieldia pusilla*, a flower originating from the Swiss Alps, was studied. For this, an at-line coupling of UHPLC-ESI-TOF/MS, micro-flow CapNMR and cancer chemopreventive assays [2] together with semi-preparative HPLC micro-fractionation was used. The dereplication of quinone reductase (a phase 2 detoxification enzyme) inducers in combination with chemometric tools [3] demonstrates the efficiency of the approach.

[1] Wolfender JL et al., *Curr. Org. Chem.* **2010**, *14* (2010) 1808.
[2] Cuendet M et al., *J. Nat. Prod.* **2006**, *69*, 460.
[3] Crockford DJ et al., *Anal Chem.* **2005**, *78*, 363.

Analytical Chemistry **38**

Trace Elemental and Isotopic Analysis of Objects in the Field by Means of a Portable Laser Ablation Sampling Device

Reto Glaus, Ladina Dorta, Detlef Günther

ETH Zürich, Laboratory of Inorganic Chemistry, Wolfgang-Pauli-Strasse 10, CH-8093 Zürich

Laser ablation inductively coupled plasma mass spectrometry (LA-ICPMS) allows for trace elemental as well as isotopic analysis of solid samples in a quasi-non-destructive manner. In order to enable LA-ICPMS for samples outside the laboratory, e.g. for archeological artifacts in museums, a portable laser ablation sampling device was developed based on a diode pumped solid state (DPSS) laser and fiber optics.

The analytical performance for determination of lead and copper isotope ratios as well as elemental fingerprinting was investigated for ancient Chinese ceramic samples. Samples were ablated with a spot size of about 100 µm and 1000 pulses and the produced aerosols were sampled on membrane filters. The sampled materials were subsequently remobilized by LA and analyzed by quadrupole ICPMS or multi collector (MC)-ICPMS for trace elements and isotope ratios, respectively. The isotope ratios determined using the portable LA sampled materials are shown to results obtained by a standard LA-MC-ICPMS setup. Furthermore trace element quantification with typical limits of detection in sub-µg/g could be achieved. The setup, sampling strategies and figures of merit will be presented.

Analytical Chemistry **39**

Ablation Behaviour of Carbon in LA-ICP-MS and the Consequences for the Internal Standard Selection for the Quantification of Carbon Containing Samples

Daniel A. Frick, Luca Flamigni, Joachim Koch, Detlef Günther*

Laboratory of Inorganic Chemistry, ETH Zürich, Wolfgang-Pauli-Strasse 10, 8093 Zürich, Switzerland.

Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICP-MS) is a well-established technique for the determination and quantification of trace elements in broad range of materials. LA-ICP-MS offers low detection limits and lower matrix effects compared to other elemental imaging techniques such as μXRF and SIMS and is thus frequently chosen to study the elemental distribution in biological, geological and materials. In contrast to geological and material science applications, where a wide range of reference materials is available, quantification in biological and medical application relies on in-house standards. For concentration determinations carbon is most commonly used as internal standard, despite the applicability of carbon is controversy discussed in the literature [1, 2].

This study investigates the formation of carbon species during LA of 12 common carbon matrices using a gas exchange device (GED) [3, 4] and membrane filters to separate gas and particle species. Evidence will be provided for a matrix composition dependence on the formation of carbon gas species and on an exclusive transport of trace elements in the particle phase. Furthermore the impact of matrix-mismatched quantification will be discussed.

[1] J. L. Todolí, J. M. Mermet, Spectrochim. Acta Part B **1998**, *53*, 1645.
[2] C. Austin, F. Fryer, J. Lear, D. Bishop, D. Hare, T. Rawling, L. Kirkup, A. McDonagh, P. Doble, *J. Anal. At. Spectrom.* **2011**, *26*, 1494.
[3] K. Nishiguchi, K. Utani, E. Fujimori, *J. Anal. At. Spectrom.* **2008**, *23*, 1125.
[4] R. Kovacs, K. Nishiguchi, K. Utani, D. Günther, *J. Anal. At. Spectrom.* **2010**, *25*, 142.

Analytical Chemistry **40**

Behavior of laser-produced aerosols in inductively coupled plasmas

Luca Flamigni[1], Joachim Koch[1], Detlef Günther[1]

[1] ETH Zurich/Laboratory of Inorganic Chemistry, Wolfgang-Pauli-Strasse 10, 8093 Zurich, Switzerland

Laser ablation inductively coupled mass spectrometry (LA-ICP-MS) is the technique of choice for the elemental analysis of solid samples. It is, in fact, the only one to offer sample imaging capabilities, quantitative isotopic analysis, atmospheric pressure sampling, low limits of detection (ppb-range) together with a fast and easy sample preparation. The matrix dependence of the analytical technique requires however a sample-matrix matched external standard material, for accurate quantitative analysis. The reason for this requirement is the size- and species-dependence of several steps of the analysis, from the laser ablation event on to the transport of the aerosol to the ICP and finally its atomization and ionization [1]. The work presented here deals with the interaction of the laser-produced aerosol particles with the plasma. Experimental data including optical emission and mass spectrometry combined with ab-initio calculations based on the works by Horner et al. [2,3] show how the chemical form of the analyte can alter the signal intensity measured by ICP-MS. This effect will, on its turn, impair the quantification if a non-matrix matched external standard material is used. The implication on the LA-ICP-MS analysis of materials is shown, particularly in the case where a matrix-matched standard reference material is not available or can readily be produced in the laboratory. Possible workarounds and mathematical correction approaches of the simulation work will be discussed.

[1] R. E. Russo, X. Mao, H. Liu and J. Gonzalez, *Talanta*, **2002**, *57*, 425.
[2] J. A. Horner , S. A. Lehn and G. M. Hieftje, *Spectrochim. Acta, Part B*, **2002**, *57*, 1025.
[3] J. A. Horner, G. C.-Y. Chan, S. A. Lehn and G. M. Hieftje, *Spectrochim. Acta, Part B*, **2008**, *63*, 217.

Analytical Chemistry **41**

First steps towards the coupling of LA and AMS: cell design and CO_2-production efficiencies of different lasers

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

[1]Laboratory of Inorganic Chemistry, D-CHAB, ETHZ, Wolfgang-Pauli-Str. 10, 8093 Zurich, Switzerland
[2] Laboratory of Ion Beam Physics, ETHZ, Schafmattstr. 20, HPK, 8093 Zurich, Switzerland

^{14}C is an important dating isotope for carbonates such as speleothems, corals and shells [1]. Therefore, using laser ablation (LA) as a sampling tool for ^{14}C-analysis of carbonate archives to make detection faster and increasing the spatial resolution is desirable. The CO_2 produced during laser ablation can directly be introduced into the gas ion source of the compact accelerator mass spectrometer MICADAS (MIniCArbonDAtingSystem) at ETH [2]. We designed an ablation cell that combines fast washout with minimal particle deposition on the cell's window and walls which enables a short measurement time and reduces cross-contamination. The cell has the ability to host large samples and is equipped with a positioning system allowing precise scanning at high spatial resolution. Furthermore the CO_2 production efficiency on carbonates of three different laser ablation systems was tested with an ICP-MS (Elan 6100): an ArF excimer laser (GeolasC, $\lambda = 193$ nm), a Nd:YAG laser (LSX213, 5ω, $\lambda = 213$ nm) and a Nd:YAG laser (LSX500, 4ω, $\lambda = 266$ nm). The production efficiency of the excimer laser is significantly higher than of the other lasers making it best suited for the coupling of LA and AMS.

[1] Libby, W.F., Anderson, E.C., Arnold, J.R., *Science* **1949**, 109, 227.

[2] Ruff, M., Wacker, L., Gaggeler, H.W., Suter, M., Synal, H.A., Szidat, S., *Radiocarbon* **2007,** 49, 307.

Analytical Chemistry **42**

Mass Bias Study on Cu and Zn using Different Introduction Systems for Multi Collector-ICPMS

Ladina Dorta, Bodo Hattendorf, Detlef Günther

ETH Zürich, Wolfgang-Pauli-Strasse 10, 8093 Zürich, Switzerland

Mass bias effects are affecting the accuracy of isotope ratio determinations by multi collector inductively coupled plasma mass spectrometry (MC-ICPMS). For accurate isotope ratio measurements, the measured intensity ratios need to be corrected for this bias. The correction is typically carried out with an isotope pair of known abundance ratio by means of empirically derived relationships of the mass-dependent isotope transmission. The isotope pair used for the correction is ideally from the same element, minimizing chemical effects. For isotope systems, which lack such an isotope pair, often inter-element correction is proposed in the literature[1,2].

This study investigates the potential of inter-element corrections for accurate Cu isotope ratio measurements using MC-ICPMS. Cu intensity ratios were measured with a Nu Plasma HR MC-ICPMS instrument (Nu Instruments, UK) and the instrumental bias was monitored and corrected using Zn isotopes. The influence of instrument settings and the sample composition on the mass bias was studied for brass reference samples with different Cu/Zn fractions. The samples were analyzed by laser ablation and, after digestion, by solution nebulization, either as desolvated or wet aerosol.

In order to test the accuracy of the method, the isotopic composition of the brass reference samples was determined against NIST SRM 976 and JMC-Zn for Cu and Zn respectively, prior to the study.

Wet introduction shows similar fractionation of the two elements, while dry sample introduction (desolvating and laser) is more sensitive to instrument settings and matrix composition. The accuracy, however, remains in the same range for all sample introduction systems.

[1] Longerich H. P., Fryer B. J., Strong D. F., *Spect. Acta*, **1987**, *42B*, 39.
[2] Maréchal C. N., Télouk P., Albarède F., *Chem. Geol.*, **1999**, *156*, 251.

Analytical Chemistry **43**

Analysis of single nanoparticles by a new Inductively Coupled Time-of-Fight Mass Spectrometer

O. Borovinskaya[1], B. Hattendorf[1], M. Tanner[2], S. Gschwind[1], D. Günther[1]

[1] ETH Zurich/Department of Inorganic Chemistry, Wolfgang-Pauli-Strasse 10, Zurich, Switzerland
[2] Tofwerk AG, Uttigenstrasse 22, Thun, Switzerland

The application field of ICPMS in nanoparticle analysis has gradually expanded [1]. Among many separation techniques which employ ICPMS as a sensitive elemental detector, direct Single Particle (SP)-ICPMS analysis using a microdroplet dispenser has been successfully applied [2]. The capacity of obtaining simultaneous information about nanoparticle elemental composition, mass and concentration makes SP-ICPMS the method of choice. However, the analysis of single multi-elemental nanoparticles represents a serious challenge for ICPMS and is beyond the capabilities of currently available instrumentation.

The acquisition of very fast transient signals, simultaneous multi-isotopic detection over the entire mass range and fast data readout are required for SP-ICPMS. To fulfill the aforementioned requirements a prototype ICP TOF mass spectrometer was set up in our laboratory by coupling an ELAN6000 plasma interface (PerkinElmer, USA) to a fast TOF mass spectrometer (TOFWERK AG, Switzerland). The analytical performance of the prototype will be shown and the capabilities of the new instrument for multi-elemental nanoparticle analysis will be discussed in detail.

[1] P. Krystek, A. Ulrich, C. C. Garcia, S. Manohar , R. Ritsema, *J Anal. At. Spectrom.* 2011, 26, 1701–1721.

[2] S. Gschwind, L. Flamigni, J. Koch, O. Borovinskaya, S. Groh, K. Niemax, D. Guenther, *J Anal. At. Spectrom.* 2011, 26, 1166–1174

Analytical Chemistry **44**

Ablation cell and sample size independent sampling strategies for LA-ICPMS using Large-Capacity Gas Exchange Devices

D. Tabersky[1], K. Nishiguchi[2], I. de Maddalena[1], R. Dietiker[1], M. Ohata[1], and D. Günther[1]

[1]ETH Zurich, Laboratory of Inorganic Chemistry, 8093 Zurich, Switzerland
[2]Sumitomo Seika Chemicals Co., Ltd., 346-1 Miyanishi, Harima-cho, Kako-gun Hyogo, 675-0145, Japan

Trace elemental distributions in natural carbonates such as stalagmites represent a precious source of information on past climate variability and can be assessed by laser ablation inductively coupled plasma mass-spectrometry (LA-ICPMS). However, recording trace element distributions of such large and bulky samples applying LA-ICPMS is a challenge. Commonly, the use of airtight ablation cells requires that larger samples have to be cut into smaller pieces to fit into such a cell. Especially for precious objects, breaking of samples is not desirable. In contrast to closed cell approaches, atmospheric ambient sampling rudiments enabled by gas exchange membranes [1, 2] allow for cell-less LA strategies independent of the size of the sample.

However, complete aerosol suction is mandatory to provide accurate results in low concentration ranges. In order to avoid aerosol losses resulting from incomplete aerosol collection, a plume entrainment device was constructed based on design optimization by computational flow dynamics (CFD). Further, in this study analytical figures of merit (limits of detection, sensitivities, signal to background ratios, oxide ratios, signal stabilities, and signal dispersion) using a new prototype membrane were investigated using glass standards and compared to closed cell approaches. Particle residence times were related to washout times of closed cells and the signal dispersion will be discussed.

[1] K. Nishiguchi, K. Utani, E. Fujimori, *J. Anal. At. Spectrom.,* **2008**, 23, 1125.
[2] R. Kovacs, K. Nishiguchi, K. Utani, D. Günther, *J. Anal. At. Spectrom.,* **2010**, 25, 142.

Analytical Chemistry **45**

Fundamental studies on LIBD

Fedotova Nataliya[a], Kägi Ralf[b], Günther Detlef[a]

[a] Laboratory of Inorganic Chemistry, ETH Zurich, 8093 Zurich

[b]Particle Laboratory, Swiss Federal Institute of Aquatic Science and Technology, CH-8600 Dübendorf

Laser induced breakdown detection (LIBD) offers an opportunity to detect particles at nanoscale in aquatic systems. This method detects individual plasma events generated by breakdown of the dielectric properties of particles that are in the high field of a pulsed and focused laser beam. Individual plasma events can be detected optically, acoustically or energetically. The breakdown probability (BDP) refers to the ratio of the number of plasma events detected to the total amount of laser pulses. Energy curves are obtained by a stepwise increase of the laser energy and recording the BDP at every energy step. The energy curves can be described by a semi-empirical model [1] with two free parameters, one of them referring to the size, the other to the concentration. The advantage of this model is that the size parameter is independent of the concentration. Our LIBD system detects plasma events by measuring the energy attenuation (energy difference of the laser pulse before and after the passage through the measurement cuvette) of every individual laser pulse. In order to calibrate our system and extract parameters such as size and concentration we used three fitting functions to describe the energy curves: logistic, asymmetric logistic and the semi-empirical model published by Walther et al. For the semi-empirical model, we used the 'Nelder-Mead simplex direct search'. In all three methods we consistently observed a concentration dependence of the size parameter (Eth), which is in disagreement with the theory of the breakdown generation of ns-laser pulses. The reason for this discrepancy could be a strongly inhomogeneous beam profile which is currently being investigated. The observed size dependence would severely limit the applicability of LIBD method to determine the size of nanoscale particles as a standalone technique.

[1] C. Walther, H. Cho, T. Fanghänel, Appl. Phys. Lett. 85 (26) (2004) 6329–6331.

Analytical Chemistry **46**

A Novel Laser Ablation Cell for Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICPMS)

H.A.O. Wang, [1, 2] Daniel Grolimund, [2] Camelia Borca, [2] James Shaw-Stewart, [3‡] Detlef Günther [1]

[1]Trace Element and Micro Analysis Group, ETH Zurich, 8093 Zurich, Switzerland
[2]microXAS Beamline Project, Paul Scherrer Institute, 5232 Villigen PSI, Switzerland
[3]Materials Group, General Energies Department, Paul Scherrer Institute, 5232 Villigen PSI, Switzerland
‡ Current address: Grant Institute, University of Edinburgh

We demonstrate in this work a novel laser ablation cell for Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICPMS). The optimized performance achieved a full width at 1% maximum of less than 50ms for transient signals of single ϕ10μm laser shots on NIST 610. Characterization of the novel ablation cell demonstrates improvements over the routinely used ablation cell, with respect to the washout time, sensitivity, and the limit of detection.

As shown in a case study, thin film patterns were laser ablated in the novel ablation cell and analyzed by sequential ICP-Quadrupole-MS and simultaneous ICP-Mattauch-Herzog-MS (Spectro GmbH). Pros and cons of both of the combinations will be compared and discussed. Prior to LA imaging, Synchrotron Radiation based micro X-ray Fluorescence (SR-microXRF) was used to measure the very same test patterns, and the results indicated that the spatial resolution and the speed of LA images are approaching values in comparison to routine SR-microXRF measurements, while still keeping its advantage of high sensitivity. Furthermore, an outlook together with some of the potential applications of the novel ablation cell will be discussed.

Analytical Chemistry **47**

The Story of Pearls – What Does Their Elemental Composition Tells Us? A comprehensive study using LA-ICP-MS

Steffen Allner, Francesca Peretti, Detlef Günther

Laboratory of Inorganic Chemistry, ETH Zurich, Wolfgang-Pauli-Strasse 10, CH-8093 Zurich, Switzerland

Pearls from bivalve molluscs are very popular as jewellery and are one pillar of the jewellery business. To cope with the increasing demand, cultured pearls are produced in contrast to 'accidental', natural pearls found in bivalves in the wild.

For value of a cultured pearl, the origin and colour are important factors. Therefore, it is a demand for an easy method to test the actual properties of a given pearl, especially, if the pearl comes from the claimed origin or was treated in certain ways to enhance the colour or to bleach it.

In this work, we analysed 119 mostly cultured pearls with partial information about the place of origin, treatment history, colour, and whether the pearl was grown in fresh or salt water. By the means of LA-ICPMS we investigated 47 elements with single spot analysis on the unprepared pearls and, exemplary, performed a mapping of a cut pearl.

Additionally to the quantitative description of elements present in pearls, we show the capabilities of elemental and trace-elemental information to categorise the pearls. Key elements for the clear distinction between fresh and salt water pearls will be discussed. For the discrimination of treated pearls silver could be identified as indicator.

The results of the mapping show that growth cycles, indicated by the barium signal, or seasonal changes in the elemental uptake of the bivalve could potentially be identified using LA-ICPMS.

Analytical Chemistry **48**

The role of water in liquid chromatography

Prashant Kumar Gupta and Markus Meuwly*

University of Basel, Klingelbergstrasse 80 Basel-4056, Switzerland

Besides its function as a solvent, water is known to play an active functional role in biological and chemical systems. However, complex systems including chromatographic systems are highly influenced by the amount of water present between the silica layers. The dynamics and ordering is different due to different interfacial regions and the intermolecular interactions at the surface, e.g. -OH groups of silica surface and the hydration sites in lipid bilayers[1]. Due to this reorganisation dynamics it becomes difficult to study such systems by standard structural techniques including X-ray or NMR spectroscopy.

Owing to the great practical relevance of reversed phase liquid chromatograpy (RPLC), a more detailed study of water dynamics, energetics and the morphology in the stationary phase and at interfaces is carried out by molecular dynamics (MD) simulations. In this work we investigate the exchange dynamics of water between the surface-region and the bulk[2] and the H-bond structures, dynamics at water/silica interface[3,4], because it selectively captures the interfacial dynamics and contribute to their stability and functionality through hydrogen bond interaction[5,6]. Such a study reveals the temporal evolution of this complex system at an atomistic level.

[1] Finer E., Darke A., *Chem. Phys. Lipids*, **1974**, *12*, 1.
[2] M. Orzechowski and M. Meuwly *J. Phys. Chem. B* **2010**, *114*, 12203.
[3] Nagata Y., and Mukamel S. *J. Am. Chem. Soc*., **2010**, *32*, 6434.
[4] J. Braun, A. Fouqueau, R. J. Bemish and M. Meuwly *PCCP* **2008**, *10*, 4765.
[5] S. M. Melnikov et. al. *Analytical Chemistry*, **2011**, *83*, 2569.
[6] L. Zhang, S. Singh, et. al. *J. Chem. Phys*., **2009**, *130*, 154702.

Analytical Chemistry **49**

Structural studies of hormone functional amyloids by NMR-based quenched hydrogen/deuterium exchange

Nadezhda Nespovitaya, Carolin Seuring, Roland Riek

Swiss Federal Institute of Technology (ETH Zurich), Wolfgang-Pauli-Strasse 10, 8093 Zurich, Switzerland

For a very long time protein amyloids have been associated with a number of devastating diseases such as Alzheimer's disease, Parkinson's disease, diabetes type II to name only a few. Amyloid depositions were found in affected organs and assigned to toxic activity. Later, the functional aspect of amyloid structures was discovered and shifted the paradigm towards potential benefits of amyloid formation in many cases (HET-S protein, CPEB, Pmel17, etc.) due to the highly stable and compact structure. Recently, a hypothesis suggesting that amyloid structure serves as a depot for peptide hormone storage was proposed. It was shown that amyloids formed by human peptide hormones participate in hormone sorting process, hormone storage in the secretory granules, and controlled release upon triggering [1].

In the present work we study amyloid fibrils formed by human β-endorphin and somatostatin14 (SS14), which are peptide hormones produced in pituitary gland and hypothalamus, respectively. We employ the DMSO-quenched H/D exchange in combination with solution-state NMR spectroscopy method to explore how hormone molecules are arranged within the amyloid fibril. In our preliminary experiments, we observed that the overall rates of exchange in the peptides packed into amyloid fibrils were significantly decreased compared to the free monomers suggesting that fibrillar structure is very compact and rigid. Moreover, rates of exchange for individual amino acid residues varied greatly. By comparing the individual exchange rates we can discriminate buried amino acid residues from those that are exposed to the environment. Our data therefore provide an insight into 3D structure of SS14 and β-endorphin amyloid fibrils.

[1] Maji SK *et al*. *Science*. **2009**, *325*(5938):328-332.

Analytical Chemistry **50**

Chemical Composition of *Phyteuma orbiculare*, a Forgotten Food Plant of Valais

Christian Abbet[1], Ivan Slacanin[2], Matthias Hamburger[1], Olivier Potterat[1*]

[1]Division of Pharmaceutical Biology, University of Basel, Klingelbergstrasse 50, CH-4056 Basel, Switzerland
[2]Ilis Institute & Laboratory, Impasse de la Passerelle 17, CH-2503 Bienne, Switzerland

Numerous wild plants have been used in the past by the local Alpine population as foods. Most of these have fallen into oblivion, and very little is known about their constituents and biological properties. In this context, we initiated a project to shortlist forgotten plants which could potentially be taken into cultivation and re-established as food plants with interesting gustatory properties and a phytochemical composition which might have beneficial effects on health. Based on an ethnobotanical survey in the lower Valais region (Switzerland) and considerations concerning the feasibility of large scale cultivation, the round-headed rampion (*Phyteuma orbiculare* L., Campanulaceae), which was eaten in the past as a salad, has been selected for in depth phytochemical investigation.

Extracts of different polarities were subjected to a comprehensive metabolite profiling using a dereplication platform combining HPLC-PDA-MS, and offline NMR analyses. Fatty acids, triterpenoids, and phenolic glycosides were identified online or after targeted isolation. A new dimeric phenylpropanoid glucoside, tangshenoside VII, and two structurally unique triterpene saponins, phyteumosides A and B [1], were characterized. In addition, substances relevant for nutrition such as β-carotene, fatty acids, ascorbic acid, and minerals were quantified in leaves and flowers. Total phenolic compounds were determined as gallic acid equivalents and antioxidant properties were assessed by an ORAC (Oxygen Radical Absorbance Capacity) assay. Finally, the study was extended to include the taxonomically closely related species *P. spicatum*, *P. hemisphaericum*, and *P. ovatum*.

[1] C. Abbet, M. Neuburger, T. Wagner et al. *Org. Lett*. **2011**, *13*, 1254.

Analytical Chemistry **51**

Binding of Platinum-Based Anticancer Drugs to Higher-Order Nucleic Acids - A Tandem Mass Spectrometric Study

Valentine Grimaudo[1], Silvan R. Stucki[1], Adrien Nyakas[2], Stefan Schürch[1]

[1]University of Bern, Department of Chemistry and Biochemistry, Freiestrasse 3, 3012 Bern, Switzerland
[2]University of Victoria, Genome Proteomics Centre, 3101-4464 Markham Street, Victoria, BC, Canada

Quadruplexes are higher-order nucleic acid structures consisting of stacked guanine quartets. They are formed by the guanine-rich telomeric DNA sequences and are believed to be involved in cellular key processes, such as cell cycle control and chromosome maintenance. Therefore, quadruplexes represent a promising target for therapeutic applications. Cisplatin, which revolutionized the anticancer therapy, was found to bind preferentially to adjacent GG base pairs in nucleic acids. The induced distortion of the double helix results in inhibition of replication as well as transcription, thus, leading to apoptosis. Though cisplatin exhibits high effectiveness against several tumor types, its range of action is not unlimited and the application of the drug is further restricted by severe side effects. Consequently, the development of alternative anticancer drugs became a key issue.

Herein we report on the elucidation of the binding behavior of 2nd and 3rd generation platinum-based compounds to duplex and quadruplex DNA by nanoelectrospray high-resolution tandem mass spectrometry.

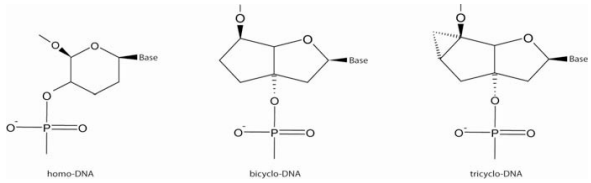
Analytical Chemistry **52**

Sequencing of Sugar-Modified Nucleic Acid Analogues by Tandem Mass Spectrometry

Silvan R. Stucki[1], Adrien Nyakas[2], Camille Désiron[1], Branislav Dugovič[1], Christian J. Leumann[1], and Stefan Schürch[1]

[1]University of Bern, Department of Chemistry & Biochemistry, Freiestrasse 3, 3012 Bern, Switzerland
[2]University of Victoria, Genome Proteomics Centre, 3101-4464 Markham Street, Victoria, BC, Canada

In recent years, nucleic acids and in particular their modified analogues have gained widespread attention for therapeutic and diagnostic applications. Modified synthetic oligonucleotides, such as bi- and tricyclo-DNA or locked nucleic acids were found to exhibit improved base-pairing properties, resulting in enhanced stability of hybrid-constructs with complementary biological DNA or RNA. On the other hand, *homo*-DNA, a hexose-modified DNA homologue does not interact with naturally occurring nucleic acids at all, therefore making it suitable for templated chemistry, imaging, and sensing applications. In the present study, high-resolution electrospray tandem mass spectrometry was applied to the structural characterization of various types of sugar-modified oligonucleotides. The sequence-defining fragment ion series generated upon collisional activation are described and the underlying mechanistic aspects of the gas-phase dissociation are discussed. It is demonstrated, that tandem mass spectrometry is a suitable tool for sequence elucidation of unnatural nucleic acid analogues.



Modified sugar moieties: homo-DNA; bicyclo-, and tricyclo-DNA.

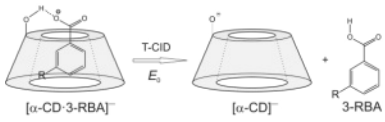
Analytical Chemistry **53**

The Quantitative Study of Intrinsic Non-covalent Interactions within Complexes of α-Cyclodextrin and Benzoate Derivatives

Zhongshu Li, Xiangyang Zhang

Laboratory of Organic chemistry, ETH Zurich, Wolfang-Pauli-Strasse 10, CH-8093 Zurich, Switzerland

Dissociation energies and structural assignments of α-cyclodextrin complexes were for the first time studied by the combination of experiments and theoretical calculations. Qualitative experiments were performed to obtain the relative stability order for several α-cyclodextrin complexes [1]. Threshold collision-induced dissociation (T-CID) experiments were performed on a customized 24-pole Finnigan TSQ–700 tandem mass spectrometer to get accurate dissociation energy [2]. DFT calculation was carried out to further interpret non-covalent interactions within host-guest complexes. Hydrogen bonding interaction, such as O–H···O, C–H···O and C–H···π interactions contribute mainly for the stability of gaseous complexes. Inclusion geometries are still favored according to the experimental and calculation results. The experimental stability order and absolute dissociation energies (Figure 1) are in excellent agreement with DFT calculation results.



R	E_{DFT} (kcal mol^{-1})	E_0 (kcal mol^{-1})
Me	40.6	40.8(5)
H	40.7	41.1(5)
OH	44.0	41.8(5)

Figure 1. Schematic view of the dissociation process, and the dissociation energies obtained from DFT calculations and T-CID experiments.

[1] Li, Z.; Couzijn, P. A.; Zhang, X. *J. Phys. Chem. B.* **2012**, *116*, 943.
[1] Narancic, S.; Bach, A.; Chen, P. *J. Phys. Chem. A.* **2007**, *111*, 7006.

Analytical Chemistry **54**

Towards a novel highly sensitive ovarian cancer diagnostic based on electrochemiluminescence detection.

Denis Prim, Elodie Baechler, Jannick Pétremand, Marc E. Pfeifer

Institute of Life Technologies, HES-SO Valais
Route du Rawyl 64, 1950 Sion

Cancers are often discovered too late when the tumor mass is critical. Mutant proteins, as BARD1, present in cancer patients have been shown to be the target of specific antibodies and represent interesting diagnostic targets. The use of peptides as bait to detect these antibodies in blood serum patients is an attractive strategy to develop new diagnostic tools. In addition, the utilization of electrochemiluminescence (ECL) as detection system was tested to improve the sensitivity and the dynamic range of the test.

The peptides can be easily synthesized and coupled to several binding partners (such as biotin) in order to immobilize them on a plate (via streptavidin). A commercially available anti-BARD1 antibody and normal serum was used as a model assay to evaluate analytical performance. In electrode-containing plates, the tagged detection antibodies are sensed after electrochemical stimulation. The use of the ECL technique with peptides immobilized on streptavidin-coated plates allows a significant improvement of the detection and quantification limits.

Indeed, the limit of detection (LOD) was improved more than 200-fold compared to a standard colorimetric immunoassay and the dynamic range achieved more than 4 Log. Our results confirm the great sensitivity of the ECL obtained with the peptides/Anti-BARD1 model assay.

Analytical Chemistry **55**

Membrane-Particle Interactions in A4F: The Importance of the Zeta-Potential in the Analysis of TiO_2-Nanoparticles

Nina Bendixen[1,2], Andrea Ulrich[1], Christian Adlhart[2], Sabrina Losert[1], Ahmed Al-Kattan[1]

[1] Empa Swiss Federal Laboratories for Material Science and Technology. Laboratory for Analytical Chemistry, Überlandstrasse 129, 8600 Dübendorf, Switzerland
[2] ZHAW Zurich University for Applied Science, Grüntal, P.O. Box, CH-8820 Wädenswil, Switzerland

Asymmetric flow field flow fractionation (A4F) is a powerful and promising technique for the separation of nanoparticles. In combination with UV/Vis detection, multi-angle light scattering (MALS) and coupled to ICP-MS a fast and quantitative analysis of particle size, size-distribution and elemental composition is possible. A central element in this setup is the A4F-membrane-particle interaction. Depending on the zeta-potentials of both the nanoparticles and the A4F-membrane more or less repulsion occurs in the flow channels due to electrostatic forces. This can influence the separation performance and the recovery rate of the particles.[1] By means of systematically evaluating the influencing parameters on the zeta-potential of TiO_2 suspensions and on the zeta-potential of A4F-membranes the basis for an A4F method development was set.

[1] A. Ulrich, S. Losert, N. Bendixen, A. Al-Kattan, H. Hagendorfer, B. Nowack, C. Adlhart, J. Ebert, M. Lattuada, K. Hungerbühler, *JAAS.* **2012,** *in press*.

Analytical Chemistry **56**

Single cell ELISA

K. Eyer, S. Stratz, P. Kuhn, S. K. Kuester, P. S. Dittrich

Department of Chemistry and Applied Biosciences, ETH Zurich

We present a microfluidic device that allows parallel single cell trapping and isolation in microchambers, repeated treatment and washing steps, subsequent lysis and analysis by enzyme linked immunosorbent assays (ELISA). The two-layer microfluidic device consists of 60 circular microchambers (volume 625 pL), each features a central cell trap. Within a few hundred ms, the content of the microchamber can be fully exchanged while the cells remain trapped. In their closed state the microchambers fully isolate the cells from surrounding solution [1].

Prior to the chip assembling, the bottom glass slide of the device is patterned by micro contact printing with binding spots as required for the ELISA. In the experimental procedure, cells are immobilized at the traps and the chambers are closed. Next, lysis buffer is introduced and the chambers are shortly opened and closed again. The trapped cells are lysed within seconds, and the released target molecules bind to the antibodies on the surface. After 15 min incubation time, the chamber is flushed with buffer to remove residual cell lysate and the enzyme (horse-radish peroxidase)-linked detection agent is added to the bound antigen. Due to the consecutive opening and closing of the microchambers, cross contamination is successfully avoided. Next, the substrate (Amplex Red) is introduced, and the emergence of the fluorescent product (resorufin) is detected in the closed microchamber by fluorescence microscopy.

The performance of the device is demonstrated in a number of studies on adherent and suspension cells; by an immunoassay directly imaging the green fluorescent protein (GFP) expressed in HEK 293 TREx cells; by direct ELISA analyzing the enzyme GAPDH in U937 cells and in a competitive ELISA format for determination of the secondary messenger cyclic adenosine monophosphate (cAMP) in MLTC cells.

[1] K. Eyer, P. Kuhn, C. Hanke, P.S. Dittrich, Lab Chip, 12 (2012).

Analytical Chemistry **57**

^{1}H HR-MAS NMR Spectroscopy to monitor the impact of different production systems on the metabolic profile of Golden Delicious apples

M. Vermathen[1], M. Marzorati[1], D. Baumgartner[2], C. Good[2], P. Vermathen[3]

[1]University of Berne, Department of Chemistry & Biochemistry, Freiestrasse 3, 3012 Berne, Switzerland
[2]Forschungsanstalt Agroscope Changins-Wädenswil ACW, Wädenswil, Switzerland
[3]University and Inselspital Berne, Department of Clinical Research (AMSM), Berne, Switzerland

In this study we present the application of ^{1}H High Resolution Magic Angle Spinning (HR-MAS) NMR spectroscopy on apple tissue to analyze the metabolic profiles of apples grown under 3 different cultivation methods. Previously, we have demonstrated the feasibility of ^{1}H HR-MAS NMR spectroscopy directly performed on apple tissue as analytical tool for metabonomic studies. [1]

Golden Delicious apples were grown applying organic (Bio), integrated (IP) and low-input (LI) plant protection strategies. A total of 70 ^{1}H HR-MAS NMR spectra were analyzed by means of principle component analysis (PCA) and partial least squares discriminant analysis (PLS-DA).

Apples derived from Bio-production could be well separated from the two other cultivation methods applying both, PCA and PLS-DA. Apples obtained from integrated (IP) and low-input (LI) production discriminated when taking the third PLS-component into account. The compounds responsible for the separation could be identified by analyzing the PLS-loadings. The present study demonstrates the potential of ^{1}H HR-MAS NMR spectroscopy of fruit tissue as analytical tool for finding markers for specific fruit production conditions like the cultivation method.

[1] Vermathen, M., Marzorati, M., Baumgartner, D., Good, C., Vermathen, P., *J. Agric. Food Chem.* **2011**, *59*, 12784.

Analytical Chemistry **58**

Identification of a kinetic model based on an orthogonal instrumental methodology (OIM) with *in-situ* and simultaneous monitoring by ATR-IR, calorimetry and FBRM

Sebastien Cap, Konrad Hungerbühler

ETH Zurich, Institute for Chemical and Bioengineering, 8093 Zurich

The fast and robust identification of a kinetic hard model (deterministic dynamic model) involving complex physico-chemical processes is a challenge. Frequently, a set of plausible kinetic models can explain to a quasi-identical level of quantitative confidence the acquired signals leading to ambiguities in the mechanism identification. The classical approach, selects a plausible kinetic model based on a series of measurement of the same nature, as e.g. the ATR-IR, and sorts the "best" model using statistical estimators such as the Akaike information criterion (AIC) or a qualitative approach based on the principle of Occam's razor (*lex parsimoniae*). Alternatively, more efficient methods allowing a fast and robust identification of kinetic models aim at monitoring the process based on an orthogonal instrumental methodology (OIM) [1].

The present contribution aims at demonstrating, through a practical example, the unique efficiency of the OIM method to discriminate between multiple plausible kinetic models of a reactive dissolution process. The presented kinetic investigations were based on the monitoring involving multiple signals and chemometric methods using a hard modeling with an implicit calibration of the linear parameters (calibration free method). The dynamic model scenarios were based on a population balance equation (PBE) where particle dissolution and breakage were simultaneously considered.

[1] Blackmond, D. G., *Angewandte Chemie*, **2005**, 44, 4302-4320.

Analytical Chemistry **59**

First, second order and multi-way determination of the Arrhenius model parameters via ATR-IR, calorimetry and FBRM: a chemometric study

Sebastien Cap, Konrad Hungerbühler

ETH Zurich, Institute for Chemical and Bioengineering, 8093 Zurich

Classically, the thermodynamic model parameters associated to a dynamic process, as e.g. the activation energy, are gathered from the Arrhenius representation where the data are treated independently and sequentially. This analysis method is referred as a first order analysis (FO). Considering, a process monitored by multiple analytical devices a FO approach, leads to different estimates of the model parameters, since un-modeled phenomena related to measurements errors are random. Consequently, a question arises: how to determine the most "correct" parameter estimate?

While the chemometric methods describing the combination of identical signal is known (second order global analysis), the association of multiple signals (multi-way analysis) is at its infancy.

This contribution aims at presenting the application of state of the art chemometric methods dedicated to the simultaneous analyzes of multiple experiments and signals of any nature and nested in a single optimization step. For this purpose, a reactive dissolution has been monitored simultaneously and *in-situ* by ATR-IR spectroscopy, calorimetry and FBRM. The "best" statistical "tread-off" parameters of the Arrhenius model were determined by the optimization of multiple objectives functions, using a controlled elitist multi-objective genetic algorithm (MOGA) [1].

[1] Gianoli, S. I., *Chemometr. Intell. Lab. Syst*., **2007,** 85, 47-62.

Analytical Chemistry **60**

Determination of actinides in spices by means of alpha spectrometry after enrichment by an electrodeposition procedure

Devraj Thimmaiah and Markus Zehringer

Kantonales Laboratorium, Kannenfeldstrasse 2, 4056 Basel

In environmental and food monitoring radionuclides are normally analysed with gamma spectrometry. But only a few nuclides of the uranium- and thorium-series can be detected with these technique. Alpha spectrometry is the method of choice for the determination of alpha nuclides in the mBq range. However, the analytes have to be separated from interfering nuclides prior to their measurement by alpha spectrometry. In a first step, samples are ashed dry at 600°C and the ashes are digested in a microwave oven. Further, the actinides in these leachates are coprecipitated with calcium phosphate and redissolved in nitric acid. The phosphate ions interfere with tetravalent actinides and affect their retention on UTEVA [1]. Therefore, phosphate has to be eliminated by precipitation with aluminium nitrate. The Th- and U-fractions are separated on an UTEVA column and are evaporated close to dryness and calcinated in presence of sodium hydrogen sulfate. The calcinated residue is redissolved in a sodium hydrogen sulfate/sodium sulfate mixture and electrodeposited [2]. The prepared sources are measured with alpha spectrometry (silicon barrier detector). The spiking of the samples with tracers allows to monitor and recalculate the chemical recoveries. Several difficulties are encountered: 1) Thorium oxalate precipitates are formed by stripping thorium with oxalic acid and, thus poor chemical recoveries were observed 2) Thorium recoveries decreased with increased amount of Thorium 3) The use of iron(II) sulfamate for the reduction of Pu dramatically reduced uranium recoveries. After some modifications the presented method could be applied successfully for spice and mushroom samples.

[1] TrisKem UTEVA product catalogue, www.triskem-international.com

[2] S. Bajo, J.Eikenberg, *J. Radioanal. Nuc. Chem.* **1999**, *242*, 745-751

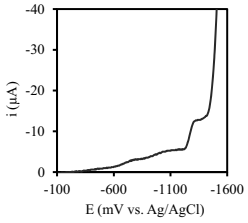
Analytical Chemistry **61**

Voltammetric Reduction of Iron Sucrose in Alkaline Solution

Leila Mahmoudi, Reinhard Kissner and Willem H. Koppenol

ETH Zürich, Wolfgang-Pauli-Str. 10, 8093 Zürich, Switzerland

Iron sucrose is an iron(III)oxide hydroxide compound, consisting of clusters which are enveloped by a layer of sucrose molecules. The original iron sucrose, Venofer, is very important in the treatment of iron deficiency anemia, due to its favorable safety profile [1].
We applied cyclic voltammetry to investigate the chemical properties of Venofer. Voltammograms at an Au electrode in 1.1 M sodium acetate, pH=8.5, were obtained. In the potential range of -100 to -1600 mV *vs.* Ag/AgCl, two major current waves are detected.



We assign the first broad wave near -700 mV to the reduction of iron(III) to iron(II). According to literature [2, 3] the second wave at -1300 mV represents the reduction of iron(II) to iron(0). However, a 2-electron transfer requires that the second wave is 2 times higher than the first, but we found a ratio of 1:1. In addition, no distinguishable second wave is seen in the voltammetric scan of a fresh iron(III) solution in acetate immediately after mixing. We conclude that both waves represent reduction of iron(III) to iron(II), but of species that differ in structure.

[1] J. E. Toblli, G. Cao, L. Oliveri, M. Angerosa, *Arzneimittelforschung* **2009**, *59*, 76-190.
[2] J. J. Lingane, *J. Am. Chem. Soc*. **1946**, *68*, 2448-2453.
[3] L. Meites, *J. Am. Chem. Soc*. **1951**, *73*, 3727-3731.

Analytical Chemistry **62**

Intact protein isoform characterization by top-down mass spectrometry

Luca Fornelli, Unige A. Laskay, Anton N. Kozhinov, Yury O. Tsybin

Biomolecular Mass Spectrometry Laboratory, École Polytechinque Fédérale de Lausanne, 1015 Lausanne, Switzerland

Mass spectrometry (MS)-based identification and characterization of intact proteins has recently received a particular attention in discovery proteomics, partly due to the fundamental limitations of the commonly employed bottom-up proteomics. Indeed, MS analysis of intact proteins allows not only obtaining information about the intact protein molecular weight but also, when gas-phase protein ion fragmentation is applied, to investigate the protein primary structure. Through this approach, known as top-down MS, it is possible to characterize both different protein isoforms (originated by gene polymorphisms or alternative splicing) and also protein species, which own specific sets of post-translational modifications (PTMs).

Here we present the experimental results obtained on a high resolution hybrid linear ion trap Orbitrap Elite Fourier transform mass spectrometer. We applied electron transfer dissociation (ETD) and higher energy collision induced dissociation (HCD) as ion activation and dissociation methods. ETD and HCD, as well as their combination, are particularly suitable for intact protein analysis as they result in extensive protein sequence coverage with reduced PTMs loss, accompanied by the cleavage of disulfide bonds.

The combination of ETD and HCD with high resolution MS is beneficial for the confident assignment of multiply-charged product ions in complex mass spectra, especially when high molecular weight proteins such as the ~150 kDa immunoglobulins G are analyzed [1][2]. Furthermore, the here reported results include an attempt towards ETD parameters optimization (ion-ion interaction time, precursor charge state selection, etc) for the challenging structural investigation of large proteins.

[1] Y. O. Tsybin, L. Fornelli, C. Stoermer, M. Luebeck, J. Parra, S. Nallet, F. M. Wurm, R. Hartmer, *Anal Chem* **2011**, *83*, 8919
[2] L. Fornelli, et al., *submitted*

Analytical Chemistry **63**

Optimization of CH_4 adsorption from ambient air for high precision isotopic analysis by laser spectroscopy

Simon Eyer[1], Joachim Mohn[1], Andreas Borgschulte[2], Hubertus Fischer[3] and Lukas Emmenegger[1]

[1]Laboratory for Air Pollution & Environmental Technology, EMPA, Dübendorf, Switzerland
[2]Laboratory for Hydrogen & Energy, EMPA, Dübendorf, Switzerland
[3]University of Bern, Switzerland

Methane (CH_4) is the second-most important anthropogenically emitted greenhouse gas. Considered per molecule its effectiveness in promoting global warming is 25 times higher compared to CO_2. Analysis of the most abundant methane isotopologues $^{12}CH_4$, $^{13}CH_4$ and $^{12}CH_3D$ can be employed to trace its biogeochemical cycle. However, the standard technique for isotopic analysis of trace gases, isotope-ratio mass-spectrometry (IRMS), is not suited for field applications [1].

In the current project we intend to apply an alternative approach based on mid-infrared absorption spectroscopy. The anticipated high precision analysis (0.1 ‰ for δ^{13}C-CH_4, 1 ‰ for δD-CH_4) should be feasible in combination with CH_4 preconcentration from several liters of ambient air. In the first stage, different adsorbent materials for efficient CH_4 preconcentration were tested, including: molecular sieves, metal-organic frameworks (MOF) and carbon nanotubes (CNT). The characterization involves BET-surface determination and isotherm measurements in a wide range of temperatures (77-373K) on a volumetric low pressure adsorption analyzer. Moreover, CH_4 recovery and adsorption/desorption kinetics of selected adsorbents were tested on an automated liquid-nitrogen free preconcentration unit [2]. The experimental results are discussed in relation to adsorbent properties as pore size, surface area, and adsorbate-surface interactions.

[1] M. Bock et al., *RCM.* **2010**, 24, 621-633.
[2] J. Mohn et al., *Atmos. Meas. Tech.*, **2010**, 3, 609–618.

Analytical Chemistry **64**

Characterization of coffee brews made from different roasting degrees of coffee using size exclusion chromatography and antioxidant assays

S. Smrke, S.E.W. Opitz, S. Petrozzi and C. Yeretzian

Zurich University of Applied Sciences, Institute of Chemistry and Biological Chemistry, Einsiedlerstrasse 31, 8820 Wädenswil, Switzerland

During coffee roasting, a large fraction of the chlorogenic acids is degraded and Maillard reaction products (also referred to as melanoidins) are formed. Besides proteins and carbohydrates also chlorogenic acids are likely incorporated in the melanoidins upon roasting, contributing to their antioxidant activity. Therefore with increasing roasting degree the antioxidant activity varies reflecting the degradation of the chlorogenic acids and the concomitant formation of melanoidins. The goal of our research is to optimize the roasting process and degree to achieve the highest possible antioxidant activity in the brew. Further, the chemical nature of the antioxidants is being analyzed to identify and characterize those fractions and chemical families with highest antioxidant potential.

Two different chromatography systems were tested to fractionate and separate coffee brew (100 % Arabica): preparative size exclusion chromatography (PSEC) and high performance size exclusion chromatography (HPSEC). After separation by PSEC the coffee fractions were collected, treated and tested for antioxidant activity with the Folin-Ciocalteau antioxidant assay. In an online assay approach, coffee brew samples were also separated with HPSEC and subsequently analyzed for their antioxidant activity.

Thereby two different types of HPSEC columns were tested, one with single pore size distribution and one with multipore particles (range of pore sizes). The different separation techniques were compared and evaluated regarding sample concentration and preparation and eluent composition. We observed similar performance in separation with both chromatography systems.

Using the Folin-Ciocalteau assays it is concluded that the fractions dominated by chlorogenic acids showed highest antioxidant activity, while melanoidin fractions gave a lower response.

Analytical Chemistry **65**

Online monitoring of the roaster off-gas during coffee roasting using proton transfer reaction time-of-flight mass spectrometry (PTR-ToF-MS): towards a real-time process control for a consistent roast profile

Flurin Wieland[1], Alexia N. Gloess[1], Marco Keller[2], Andreas Wetzel[2], Stefan Schenker[2], Chahan Yeretzian[1]

[1]Zurich University of Applied Sciences, Institute of Chemistry and Biological Chemistry, 8820 Wädenswil, Switzerland
[2]Bühler AG, Gupfenstrasse 5, 9240 Uzwil, Switzerland

The flavour of a freshly prepared cup of coffee is the final expression of a long chain of transformations. Along this journey from the seed to the cup, roasting is without doubt the most significant processing step.

Here we report on the development of a fast on-line process control technology for a consistent roast, batch after batch. It involves the on-line monitoring of the roaster off-gas using Proton-Transfer-Reaction Time-of-Flight Mass-Spectrometry (PTR-ToF-MS) and the analysis of the mass spectral profiles via principle component analysis (PCA). This allows predicting the roast degree in real time. The PCA was calibrated in advance with a large number of roasting trials, to develop the predictive model. The 3D space of the three first principal components to predict in real time the roast degree, based on PTR-ToF-MS profiles of the roast gas is shown in the Figure.

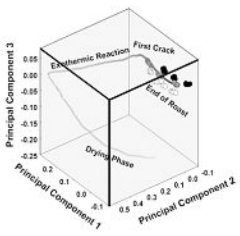


Figure: 3D space of the three first principal components. The points represent roasting experiments with a high, medium low roaster gas temperature and different roasting degrees. The line shows an actual measured roasting experiment with a medium hot-air temperature.

This work demonstrates that time resolved analysis of roaster off-gas provides a detailed picture of the evolution of the roasting process.

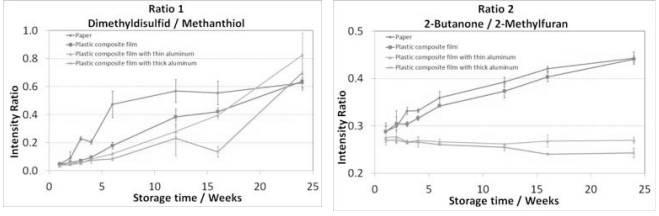
Analytical Chemistry **66**

How can we Measure the Freshness of Coffee?

Barbara Schönbächler[1], Alexia N. Gloess[1], Karin Chatelain[2], Chahan Yeretzian[1]

[1]Zurich University of Applied Science, Institute of Chemistry and Biological Chemistry, 8820 Wädenswil, Switzerland
[2]Zurich University of Applied Science, Institute of Food and Beverage Innovation, 8820 Wädenswil, Switzerland

With the growing demand for specialty coffee and the rising consumer awareness of quality in the cup, it is becoming increasingly important to establish objective and quantitative measures of coffee quality. A series of "freshness indices" were evaluated, two of which are shown below (packaged whole beans, 250 g bags). This includes specific ratios between volatile compounds that are either typical for freshly roasted coffee or an expression of degradation and oxidation processes, analyzed by HS GC-MS. We report on results during storage for up to one year, for a range of commercial single serve capsule systems (ground coffee), as well as for whole roasted beans in packaging with different barrier properties.



Ratio 1 is indicative of degration processes that occur irrespective of the barrier properties of the packaging while Ratio 2 is dependent on the barrier properties of the packaging material.

Parallel sensory measurements are in line with these instrumental results.

Analytical Chemistry **67**

Monitoring Indoor Air Quality

Kaja Knöpfli Lengweiler, Alexia N. Gloess, Barbara Schönbächler, Chahan Yeretzian

Zurich University of Applied Science, Institute of Chemistry and Biological Chemistry, 8820 Wädenswil, Switzerland

People spend on average 80 to 90 percent of the day indoor. It is therefore important to understand what is the quality of the air that we breathe in these spaces?

We have recently developed a GC-MS method for the analysis of indoor air samples that were collected on sorption tubes exposed for several hours to the atmosphere of the respective rooms. It consists in the thermal desorption from sorption tubes followed by cryogenic focusing at the inlet of the GC capillary, GC-separation and MS detection.

Here we discuss the analytical methodology and review recent results.

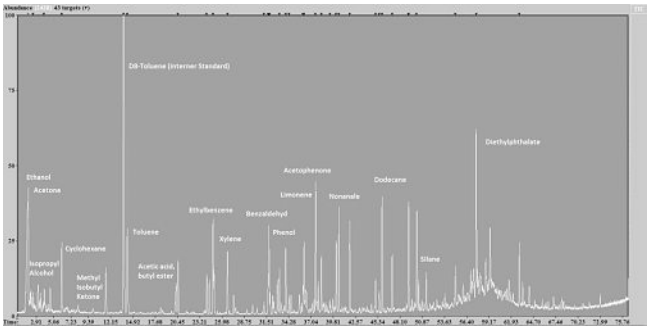


Figure: Gas Chromatogram of a typical indoor air sample.

Analytical Chemistry **68**

LC-MS/MS Profiling of Flavonoid Composition and Assessment of Anti-oxidant Capacity of twenty four different Bamboo Species

Timm Hettich[1], Jenny Dold[1], Andre Büttler[1], C. Gerig[2], Götz Schlotterbeck[1]*

[1]University of Applied Sciences Northwestern Switzerland, Institute of Chemistry and Bioanalytics, Gründenstr. 40, CH-4132 Muttenz

[2]Xibambam, Bleicheweg 3, CH-9403 Goldach

There is a worldwide growing interest in bamboo for food and health-related applications because it provides a rich source of anti-oxidants. It has been shown recently, that flavonoids and phenolic acids are the two main polyphenolic classes present in bamboo leave extracts exerting potent antioxidant capacity [1].

We present a LC-MS/MS based profiling of bamboo leaves of twenty four different species cultivated and harvested in Switzerland by assessing the flavonoid composition. In addition anti-oxidative properties of the bamboo species were investigated by ORAC [2] and DPPH [3] assays.

Bamboo species were classified on basis of their anti-oxidative capacity and individual flavonoid composition by application of chemometric data analysis methods.

[1] L. Van Hoyweghen, T. De Beer, D. Deforcea, A. Heyericka, *Phytochem. Anal.* **2012**, DOI: 10.1002/pca.1377.

[2] B.X. Ou, M. Hampsch-Woodill, R.L. Prior, *J. Agric. Food Chem.* **2001**, *49*, 4619.

[3] P. Molyneux, *J. Sci. Technol.* **2004**, *26*, 2.