doi:10.2533/chimia.2009.461 Chimia 64 (2009) 461–477 © Schweizerische Chemische Gesellschaft

SCS Grammaticakis-Neumann Prize Lecture **1**

Selective Light Induced Reactions In Solution and In Water Soluble Nano-containers

J. Sivaguru

Department of Chemistry and Biochemistry, North Dakota State University, 1231 Albrecht Blvd., Fargo, ND 58105. USA

The presentation will highlight two aspects:[1-4] a) Supramolecular Catalysis with water-soluble nano-cavities; and b) Asymmetric phototransformations in solution. This first part focuses on employing water-soluble nano-containers known as Curcurbit[8]uril (CB[8]) to control the [2+2] photodimerization of coumarin derivatives in water. The presentation will focus on the plausible reasoning for the observed photoproduct selectivity; highlight the dynamic behavior of host-guest complexes in solution and novel applications of these water-soluble nano-containers for supramolecular catalysis. The second part of the talk will focus on traditional challenges in photochemistry *viz.,* Asymmetric phototransformations in solution. Conventional chiral auxiliaries employed in ground state thermal reactions are not effective to control chirality in phototransformations, as the excited states are short-lived and there is no energy barrier for the reaction. Our approach to the problem is to use molecularly chiral chromophores, which introduce a chiral bias to the reaction pathway, leading to high selectivity in the photoproduct. The presentation will highlight our ongoing investigations where we have achieved >90% enantiomeric excess in the product in solution at ambient conditions in various light induced transformations in solution.

References

[1] Ayitou, A. J.; Jesuraj, J. L.; Barooah, N.; Ugrinov, A.; Sivaguru, J. *J. Am. Chem. Soc.,* **2009**, *131* (32), 11314–11315.
[2] Ayitou, A. J.; Sivaguru, J. *J. Am. Chem. Soc.,* **2009**, *131*(14), 5036-5037.
[3]. Pemberton, B. C.; Barooah, N.; Srivatsava, D. K.; Sivaguru, J. *Chem. Commun*. **2010**, *46*, 225 – 227
[4] Barooah, N.; Pemberton, B. C.; Johnson, A. C.; Sivaguru, J. *Photochem. Photobiol. Sci*. **2008**, *7*, 1473-1479.

Paracelsus Lecture **2**

New Tools for Molecule Makers: Enabling Technologies

Professor Steven V. Ley

Department of Chemistry, University of Cambridge, Lensfield Road, Cambridge CB2 1EW

svl1000@cam.ac.uk, http://leyitc.ch.cam.ac.uk/, http://leygroup.ch.cam.ac.uk/

The search for new ways to assemble molecules continues to be an important driver for organic synthesis. The biological activity and exquisite structural diversity of many natural products stimulates invention by challenging today's synthetic methodology. However, preparing such materials from small and commercially available building blocks inevitably involves more than one synthetic step. For most modern drugs and other complex molecules, it is not uncommon for syntheses to require at least 10 steps, and sometimes many more.

In order to make molecules more efficiently and economically, our group has developed and used solid-supported reagents in a multi-step fashion without the use of conventional work-up procedures. Now we have extended these concepts to make use of advanced scavenging agents and catch-and-release techniques, and combined these with the use of continuous flow processing to create even greater opportunities for organic synthesis.

As important examples of these developments, we have recently completed the syntheses of the natural products grossamide and oxomaritidine entirely by using these flow chemistry methods. The syntheses required the construction of a fully automated continuous flow reactor system (using a simple pumping arrangement) with immobilized reagents packed in columns to effect the synthesis steps efficiently. These examples illustrate the rapid and flexible nature of the methods for preparing compounds on demand and at various scales. The future vision of this emerging field could well cause a paradigm shift in the way chemical synthesis is conducted.

Analytical Chemistry, Lecture **3**

Capillary electrophoresis immunoassay using a multiplug magnetic beads trapping system

Anne-Laure Gassner[1], Jacques Morandini[2], Jacques Josserand[1], Hubert H. Girault[1]

[1]Laboratoire d'Electrochimie Physique et Analytique, Ecole Polytechnique Fédérale de Lausanne, Switzerland

[2]Astek Rhône-Alpes, 1 Place du Verseau, 38130 Echirolles, France

Magnetic beads (MBs) have now proven to be a powerful tool in both research and biomedical applications. They are available in a wide range of sizes and their surface can be modified with molecules having biological specificities and functions [1]. In microfluidics, MBs offer many advantages, like an increased specific surface available for molecule adsorption, a reduced diffusion pathway and easy manipulation by magnets [2].

This investigation presents a multiplug trapping system for MBs in a capillary. The capillary is inserted through a chain of cylindrical permanent magnets alternating with cylindrical non-magnetic spacers. The magnets and spacers are simply placed on the capillary like pearls on a string. The magnets are indeed drilled along their magnetization axis, parallel to the capillary and can be placed either in attraction or in repulsion. This system has the advantage of a very simple assembly. Moreover the axial symmetry and the proximity of the magnets and capillary produce high magnetic forces, giving the opportunity to work at higher flow velocities than with setups classically made up by two magnets spaced by 1 mm with their magnetization perpendicular to the capillary. Finally it is possible to adapt the number of magnets to the desired number of plugs, increasing in a controllable manner the surface available for protein adsorption in the case of an immunoassay.

[1] Pamme N, *Lab Chip*. **2006**, *6*, 24.
[2] Gijs M.A., *Microfluid Nanofluid*. **2004**, *1,* 22.

Analytical Chemistry, Lecture **4**

Applications of Proton-Transfer-Reaction Time-of-Flight Mass-Spectrometry (PTR-ToF-MS) in Coffee Research

Chahan Yeretzian, Alexia N. Gloess , Flurin Wieland

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

Over the last two years, an extensive analytical research program is being developed at the Institute of Chemistry and Biological Chemistry (ICBC) at the ZHAW in Wädenswil, one research focuses being the science of coffee. Here we report on one aspect of this program: the application of **P**roton **T**ransfer **R**eaction **T**ime-**o**f-**F**light **M**ass-**S**pectrometry (PTR-ToF-MS) in coffee research.

PTR-ToF-MS is a rapid and non-invasive analytical technology for the on-line monitoring of volatile organic compounds (VOC), with a very high mass resolution (m/Δm up to 8000), fast spectrum acquisition (complete spectrum with ppt sensitivity in less that one second) and an extended mass range (up to 50.000 amu).

We will present and discuss recent studies on the on-line monitoring of VOC during the coffee roasting process, the nose-space analysis during drinking of coffee as well as the measurement of the air-water partition coefficients (and their temperature dependence) of coffee flavour compounds.

[1] Lindinger, C.; Yeretzian, C.; Blank, I.; *Chimia*, **2009**, *63(5)*

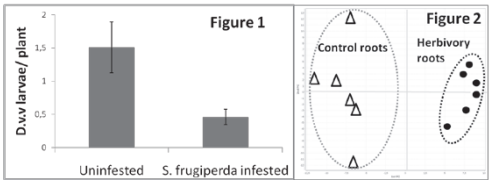
Analytical Chemistry, Lecture **5**

The response of maize roots to leaf herbivory by *Spodoptera spp.*: a metabolomic approach

Guillaume Marti[1], Matthias Erb[2], Julien Boccard[1], Serge Rudaz[1], Ted Turlings[2], Jean-Luc Wolfender[1]

[1]School of Pharmaceutical Sciences, University of Geneva, University of Lausanne, CH-1211 Geneva 4, Switzerland
[2] Laboratory for Fundamental and Applied Research in Chemical Ecology, University of Neuchâtel, Neuchâtel, Switzerland

Attack of maize leaves by the caterpillars of *Spodoptera frugiperda* negatively affects the performance of the root herbivore *Diabrotica virgifera* (Fig. 1). This implies that systemic changes induced by leaf-feeding increase the plant's root resistance. In order to understand the mechanism of this response at the metabolome level, an extended metabolomic study has been initiated. The analytical strategy involves the use of hyphenated techniques such as ultra-high liquid chromatography-mass spectrometry and atline capillary nuclear magnetic resonance (CapNMR) [1]. Several extraction procedures have been tested. Preliminary results of pooled samples from of herbivory and control roots of maize enabled a first discrimination based on the data mining of the fingerprinting results (Fig. 2). Related biomarkers will by characterized by CapNMR.



Acknowledgments: NCCR Plant Survival program is thanked for funding.
[1] G. Glauser et al. *J Chromatogr A* **2008**, 1180, 90.

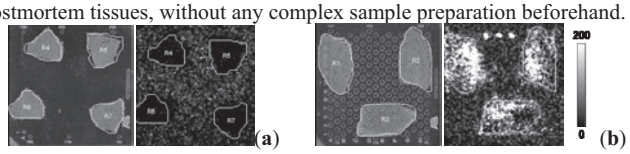
Analytical Chemistry, Lecture **6**

MALDI Mass Spectrometric Imaging in Forensic Sciences

Tiffany Porta[1], Thomas Kraemer[2], Emmanuel Varesio[1] and Gérard Hopfgartner[1]

[1]School of Pharmaceutical Sciences, University of Geneva, University of Lausanne, Quai Ernest Ansermet 30, 1211 Geneva 4, Switzerland
[2]Institute of Legal Medicine, University of Zurich, Winterthurerstr. 190/52, CH-8057 Zurich, Switzerland

To date, many approaches based on mass spectrometry (MS) coupled with HPLC or GC have been successfully developed to determine drugs of abuse in various biomatrices (oral fluid, plasma, blood, urine, hair [1]), but several steps for the sample preparation are frequently needed [2].
Previously focused on proteins/peptides [3], pharmaceuticals and their metabolites distribution for toxicology studies [4,5], MS-imaging is presented herein as a rapid and direct tissue analysis for forensic investigation on postmortem tissues, without any complex sample preparation beforehand.



(**a**) (**b**)

Fig.1: SRM/MS images of MG in negative (**a**) and positive (**b**) samples

Morphine-glucuronide (MG) was determined in human kidney slices using the selected reaction monitoring (SRM) detection mode, traducing morphine intake. An MS image (44x44 mm – pixels=0.5mm) was acquired in 40 minutes. The overall process from tissue cutting to MS images (both positive and negative samples) took less than 3 hours.

[1] Steen, H, *J. Sep. Sci.* **2009**, *32*(5-6): 825.
[2] Kraemer, T, *et al.*, *Anal. Bioanl. Chem*. **2007**. *388*(7): 1415.
[3] Caprioli, R, *et al.*, *Anal. Chem.* **1997**. *69*(23): 4751.
[4] Hopfgartner, G, *et al*., *RCM*. **2009**. *23*(6): 733.
[5] Stoeckli, M, *et al.*, A, *Int. J. Mass. Spectrom*. **2007**. *260*(2-3): 195.

Analytical Chemistry, Lecture **7**

Full Spectroscopic Mapping by Tip-Enhanced Raman Scattering in a Gap-Mode Configuration

J. Stadler, T. Schmid and R. Zenobi

Department of Chemistry and Applied Biosciences, ETH Zurich, Wolfgang-Pauli-Strasse 10, 8093 Zürich, SWITZERLAND

We present full spectral Raman imaging using a new, advantageous illumination scheme for tip-enhanced Raman spectroscopy (TERS) in a gap-mode configuration with an on-axis illumination of the tip. This illumination confines the light to a tight focus around the tip end (using a high numeric aperture, long working distance objective), thus diminishing the confocal signal from the background in TERS measurements. An on-axis illumination allows for a better defined beam profile and simultaneous top view white light images from the sample surface through the same objective.

The instrument is a combination of a confocal laser microscope with an SPM system, which allows us to keep the entire functionality range of both systems. In combination with a state-of-the-art scanning tunneling microscopy (STM) system for distance control, we show not only point spectroscopy but for the first time spectroscopic imaging with full spectral information in every pixel - necessary for the chemical identification of sample constituents. These Raman maps can be recorded with a resolution of less than 20 nm, and due to the high enhancement ($\sim 5 \times 10^6$), short acquisition times (fractions of seconds) and reasonably low illumination laser powers (in the μW regime) are possible. Thus this configuration allows a favorable compromise between resolution, time consumption and minimized thermal sample strain from the illuminating laser.

The further development and optimization of setups along with the development of reproducible, batch fabricated, highly enhancing and stable tips will be necessary for TERS to take further steps in user laboratories for routine use. We expect the development of this setup to allow a wider spectrum of possible applications and experiments, allowing users to map chemical concentrations of different chemicals on surfaces with very high resolution.

Analytical Chemistry, Invited Lecture **8**

Exploring the target and non-target analyte screening capabilities of high resolution mass spectrometry for natural water samples

Heinz Singer, Philipp Longree, Susanne Kern, Juliane Hollender

Eawag, Swiss Federal Institute of Aquatic Science and Technology

As the discovery of new emerging contaminants in the aquatic environment based on consumption data and fate assessment is often very time consuming we tested the possibility to obtain data about relevant water contaminants directly from high resolution mass spectrometric measurements of natural water samples. For this non-target analyte screening we performed measurements with an LTQ-Orbitrap running in full scan acquisition mode at a resolving power of 60'000 supplemented with data-dependent MSMS experiments. Before MS detection, enriched water samples were separated with liquid chromatography. For the identification of non-target analytes a multi-stage filtering procedure for the MS data was tested. The procedure consists of a compound detection by accurate mass screening followed by an elemental formula fit and a data bank search for the proposition of potential chemical structures. We will show, based on three illustrative examples of newly detected compounds, what the opportunities and limitations of such an approach are.

Analytical Chemistry, Invited Lecture **9**

Using Field Asymmetric Ion Mobility for conformer selection in cold ion spectroscopy

Georgios Papadopoulos, Annette Svendsen, Oleg V. Boyarkin and Thomas R. Rizzo

Laboratoire de Chimie Physique Moléculaire, Ecole Polytechnique Fédérale de Lausanne, CH-1015 Lausanne, Switzerland

As the size of biological molecules increases, their spectroscopic characterisation is hindered by the complexity of the spectra. A major source of this complexity comes from the presence of multiple stable conformations that have different but partially overlapping electronic spectra. Therefore, seperating the conformers before performing spectroscopic measurements would simplify the spectrum and allow the extension of spectroscopic techniques to much larger molecules.

Toward this end we use Field Asymmetric Ion Mobility Spectrometry (FAIMS) as a filter to select certain conformations of peptides before putting them into a cold 22-pole ion trap for spectroscopic analysis. FAIMS separates conformers on the basis of the difference of their ion mobility at high and low electric field [1]. Previous work using H/D exchange has demonstrated that FAIMS is able to separate conformers of doubly charged bradykinin [1, 2]. We present here our most recent results that prove the ability of FAIMS to simplify the electronic spectrum of doubly charged, cold bradykinin by separating its conformers at room temperature. We also demonstrate the potential of cold ion spectroscopy to provide insight into some characteristics of a FAIMS system of cylindrical geometry, when used as a conformational filter.

[1] A. A. Shvartsburg, F. M. Li, K. Q. Tang, and R. D. Smith, Anal. Chem. **78**, 3706 (2006).
[2] R. W. Purves, D. A. Barnett, B. Ells, and R. Guevremont, Rapid Commun. Mass Spectrom. **15**, 1453 (2001).

Analytical Chemistry, Lecture **10**

Applying excitation modulation X-ray absorption spectroscopy to understand sulphur poisoning-regeneration mechanisms in the biogas methanisation process

Christian Koenig[1], Davide Ferri[2], Tilman Schildhauer[1], Serge Biollaz[1], Maarten Nachtegaal[1]

[1]Paul Scherrer Institut, General Energy Departement, Villigen, Switzerland
[2]EMPA, Dübendorf, Switzerland

A biogas methanisation process has been co-developed by PSI and a commercial plant has recently been installed in Güssing, Austria. Sulphur poisoning of the catalyst is one of the major problems facing the methanisation process. By cycling the catalyst through the reactor, i.e. so that it sees different gas compositions and temperatures, sulphur poisoning can be reduced. We developed a unique experimental setup that is capable of measuring X-ray absorption spectra of a catalyst under the methanisation reaction, i.e. by reproducing the measured [1] local gas composition in the reactor. This "moving observer" setup enables us to determine the local geometric and electronic structure of a catalyst and its neighboring atoms at every point in the reactor under real conditions.

To improve the sensitivity of the extended X-ray absorption fine structure (EXAFS) spectra towards the catalyst's active surface sites, the excitation modulation spectroscopy approach [2] is applied. In combination with the moving observer setup this allows us to study the deactivation / regeneration processes in-situ, with a high sensitivity.

[1] J. Kopyscinski, T. Schildhauer, F. Vogel, S. Biollaz, A. Wokaun, *Journal of Catalysis*, **2010**, doi: 10.1016/j.jcat.2010.02.008
[2] U. Fringeli, D. Baurecht, H. Günthard, *in: Infrared and Raman spectroscopy of biological materials.* **2001**

Analytical Chemistry, Lecture **11**

Quantification of protein by MALDI-SRM/MS using chemically-assisted peptide fragmentation.

Antoine Lesur, Emmanuel Varesio and Gérard Hopfgartner[1]

[1]Life Sciences Mass Spectrometry, School of Pharmaceutical Sciences, University of Geneva, University of Lausanne, Geneva, Switzerland.

Absolute quantification of proteins with matrix-assisted laser desorption/ionization (MALDI) using the selected reaction monitoring (SRM) detection mode is demonstrated. Combination of the SRM selectivity of a triple quadrupole linear ion trap mass spectrometer with the high-throughput capability of a high repetition laser MALDI source, allows rapid and versatile samples analysis. MALDI typically generates singly-charged peptides which poorly fragment under low energy collision induced dissociation (CID) conditions. Consequently, the 4-sulfophenyl isothiocyanate (SPITC) was used as a peptide fragmentation enhancer derivatization agent [1] for low energy CID. Our results, based on a human monoclonal antibody quantitation after a tryptic digestion, show a linear response over 2.5 orders of magnitudes from 37.5 to 15'000 ng/μl (estimated concentration on spots ranges from 8 to 3'250 femtomoles).

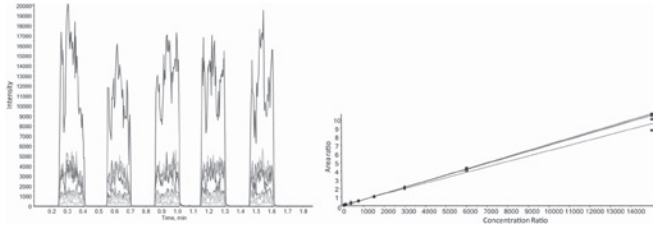


Illustration of SRM traces and calibration curve generated for four distinct tryptic peptides from the monoclonal antibody.

[1] Keough, T.; Youngquist, R. S.; Lacey, M. P. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 7131-7136.

Analytical Chemistry, Lecture **12**

On- and off-line measurement of underwater-generated laser aerosols by liquid-sampled ICP-MS

Luca A. Flamigni, Joachim Koch, Helmar Wiltsche, Detlef Günther

ETH Zurich, LAC, Wolfgang-Pauli Strasse 10, 8093 Zurich, Switzerland

Laser ablation (LA) in liquids has already been proven a valuable approach for off-line analysis with conventional liquid nebulization systems for Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) [1]. A similar strategy for the analysis of solid samples was investigated both on-line and off-line with Inductively Coupled Plasma Mass Spectrometry (ICP-MS). The new setup consisted of a small-volume cylindrical plastic ablation cell with a top glass window. To evaluate the analytical capabilities, LA ICP-MS analyses of brass samples were performed both on-line and off-line (stopped flow and continuous flow). For sample transport, a rotary peristaltic pump was placed before or, alternatively, after the cell and purified water was used as transport medium, thus keeping the ablation cell always filled with liquid. Various cell geometries and positioning were tested in order to minimize formation of gas bubbles and scattering of the laser beam before the target. Due to the appearance of frequent spikes in the MS signal, which are commonly attributed to the presence of microscopic (>1 µm) solid particles in the aerosol, a fast off-line digestion system was tested. This system consisted of a small (3 ml) stirred reactor, where an aqueous solution of nitric acid was mixed to the solution sampled from the ablation cell. A decrease in the intensity of these spikes could be achieved this way, for copper and zinc from a brass alloy sample. Furthermore, inspection of the ablation craters revealed no deposits around the walls, suggesting a potentially more representative sampling compared to gas-sampled LA-ICP-MS.

[1] E. V. Muravitskaya, V. A. Rosantsev, M. V. Belkov, E. A. Ershov-Pavlov and E. V. Klyachkovskaya, *Spectrochim Acta B* **2000**, *64*, 119.

Analytical Chemistry, Lecture **13**

A Microarray Screening for colorimetric Anion Sensors

Christine Männel-Croisé, Christian Meister, Felix H. Zelder

Institute of Inorganic Chemistry, University of Zurich, Winterthurer Str.190, 8057 Zurich

In the last few years anion recognition chemistry has focused on selective optical and luminescent chemosensors due to their simple and fast application in chemical, biological and environmental processes.[1]

One of the most popular approaches for the development of highly selective and sensitive anion chemosensors is the decomplexation of a transition metal-indicator complex by the anion of interest.[2] A higher stability constant of the transition metal–anion complex and an accompanied optical color change by the decomplexation reaction are necessary for a potential new chemosensor system.

$$Me^{2+} + \text{indicator} \rightleftharpoons Me^{2+}\text{-indicator-complex} \overset{+X^-}{\rightleftharpoons} [MeX_n]^{2-n}$$

We performed a microarray screening of metal indicators and transition metal ions. Out of over hundred combinations we selected the three most promising systems and studied them in more detail. These chemosensors were applied towards the detection of endogenous biological cyanide.[3]

[1] Cho, D.-G., Sessler, J. L., *Chem. Soc. Rev.* **2009**, *38*, 1647-1662.

[2] Xu, Z., Chen X., Kim, H. N., Yoon, J., *Chem. Soc. Rev.* **2010**, *39*, 127-137.

[3] Männel-Croisé, C.; Probst, B.; Zelder, F. H. *Anal. Chem.* **2009**, *81*, 9493-9498.

Analytical Chemistry, Lecture **14**

Fast washout laser ablation cell with circular gas flows

Mattias B. Fricker[1], Helmut Lindner[2], Detlef Günther[1]

[1]Laboratory of Inorganic Chemistry, ETH Zurich, Wolfgang-Pauli-Strasse 10, 8093 Zurich, Switzerland
[2]Department of Chemistry, University of Antwerp, Universiteitsplein 1, 2610 Antwerp, Belgium

Laser Ablation-Inductively Coupled Plasma-Mass Spectrometry (LA-ICP-MS) is a powerful technique for quantitative trace element analysis. Among other parameters, the design of the ablation cell is crucial for high spatial resolution analysis and element mapping. Minimum signal overlapping becomes more and more important for 2-D or 3-D reconstruction of elemental maps of heterogeneous samples.
Various fast-washout cells with low cell volumes for hosting samples have been reported. Fastest washout reported so far is in the order of 100 ms, which allows resolving signals generated at a laser frequency of 10 Hz [1,2]. Most of these cells have been derived experimentally.
This study was focused on the investigation of a cell that has been simulated [3] based on a previously reported so-called HEAD cell [4]. The simulation experiments indicated that minor geometrical changes could lead to a washout in the order of few tens of ms. Such an ablation cell was built in our workshop and tested experimentally. The design comprises two gas inlets and two separated sub-chambers, connected through a 300 µm orifice. The formed aerosol is carried by a low flow (He) through the orifice. The second gas provides a circular flow to minimize particle-wall interaction and to increase washout efficiency. Details about the configuration, the experimentally determined washout times in dependence on the laser frequency will be discussed.

[1] E. L. Gurevich and R. Hergenröder, *JAAS*, **2007**, *22*, 1043-1050
[2] W. Müller, M. Shelley, P. Miller, S. Broude, *JAAS*, **2009**, *24*, 209-214
[3] H. Lindner, D. Autrique, J. Pisonero, D. Günther, A. Bogaerts, *JAAS,* **2010**, *25*, 295-304.
[4] J. Pisonero, D. Fliegel and D. Günther, *JAAS*, **2006**, *21*, 922 – 931

Analytical Chemistry, Lecture **15**

Infrared functional spectromicroscopy in living cells

Luca Quaroni[1], Theodora Zlateva[1], Elise Normand[2], Kira L. Goff[2,3], Ken E. Wilson[3]

[1] Swiss Light Source, Paul Scherrer Institut, 5232 Villigen PSI, Switzerland; [2]Canadian Light Source, Saskatoon, SK, S7N 0X4, Canada; [3]Department of Biology, University of Saskatchewan, Saskatoon, SK, S7N 5E2, Canada

Fourier-transform infrared (FTIR) spectroscopy is an informative technique for the study of biological molecules, providing insight into both molecular structure and reaction mechanism. One limitation of current FTIR structure-function studies is that they often rely on purified preparations of biomolecules. In contrast, it is desirable to perform structure-function studies directly in the native milieu of a living cell, avoiding both complex purification procedures and experimental artifacts due to an environment which is far removed from the native one.

The use of a synchrotron infrared light source in place of the conventional globar source can ease this limitation thanks to its increased brightness. [1] Our work shows that the high signal-to-noise provided by synchrotron FTIR spectromicroscopy allows time-resolved measurement and identification of small-molecule metabolites and cofactors in single eukaryotic cells, including unicellular algae, retinal rod cells and fibroblast cells, and their subcellular compartments. [2] The conditions for these experiments challenge the performance of synchrotron FTIR spectromicroscopy in terms of sensitivity, spatial resolution and time resolution, and provide information on the achievable limiting performance of the technique for time resolved studies. Finally we discuss possible strategies for data analysis to extract and analyze weak absorbance data in a complex and evolving spectral background.

[1] Jamin, N.; Dumas, P.; Moncuit, J.; Fridman, W.-H.; Teillaud, J.-L.; Carr, G. L.; Williams, G. P., *Proc. Natl. Acad. Sci. U. S. A.,* **1998**, *95*, 4837.
[2] Quaroni, L., Zlateva, T., Bedolla, D., Massaro, S., Torre, V., *ChemPhysChem,* **2008**, *9*, 1380; Goff, K.L., Quaroni, L., Wilson, K.E., *Analyst*, **2009**, 134, *2216*.

Analytical Chemistry, Lecture **16**

Gold Nanostructures For Surface-Enhanced Raman Spectroscopy

Pierre Brodard, Mikhael Bechelany, Jamil Elias, Laetitia Philippe, Martin Jenke, Ivo Utke and Johann Michler

EMPA, Swiss Federal Laboratories for Materials Testing and Research, Feuerwerkerstrasse 39, CH-3602 Thun

Surface-enhanced Raman spectroscopy (SERS) is a powerful technique for identification of molecules due to the unique set of vibrational modes of any molecular species combined with tremendous signal enhancements observed on the surface of noble metals.[1] The SERS effect is essentially due to the excitation of localized surface plasmons by light. Therefore, synthetic methods allowing a complete control of the morphology and nature of metallic nanostructures are needed. Two types of fabrication techniques are presented: direct-write methods (focused electron beam-induced deposition, FEBID) and natural lithographies (electrochemical deposition). The best control over morphology is achieved by FEBID with gold precursors inside a scanning electron microscope (SEM), but this process is serial in nature and therefore results in a relatively low throughput.[2] On the other hand, natural lithography is massively parallel and allows the fast gold nanostructuring of large samples, however at the cost of a perfect uniformity of the nanostructures.[3] Here, we have systematically investigated the SERS efficiency of both types of nanostructures by covering them with a layer of organic molecules (p-mercaptoaniline or brilliant cresyl blue), allowing us to determine the best procedure to fabricate highly efficient SERS substrates for various applications.

[1] M. Fleischmann, P. J. Hendra, A. J. McQuillan, *Chem. Phys. Lett.* **1974**, *26*, 163.
[2] I. Utke, P. Hoffmann, J. Melngailis, *J. Vac. Sci. Technol. B* **2008,** *26*, 1197.
[3] M. Bechelany, P. Brodard, L. Philippe, J. Michler, *Nanotechnology* **2009**, *20*, 455302.

Analytical Chemistry **17**

Mechanistic Study of Heterogeneous Systems with Insitu Detection, Chemometrics and Fluid Flow Modelling

Sebastien Cap*, Yorck-Michael Neuhold, Miroslav Soos, Konrad Hungerbühler

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

The mechanistic-kinetic study of reactive solid-liquid systems is intrinsically complex. The modelling of a population of particles may comprise reactive-convective-diffusive terms to describe the mass flow at the particles vicinity and physical events such as their breakage. In liquid phase, time resolved spectroscopy such as ATR-IR, combined with chemometric methods [1], is often used to determine kinetic and thermodynamic parameters for a postulated kinetic model. However, as ATR-IR spectroscopy is sensitive to the absorbing chemical species in solution, the solid phase can only be indirectly described.

We have developed a well characterised and controlled reactor [2] to study heterogeneous systems. It allows simultaneous insitu ATR-IR spectroscopic and heat flow monitoring, and, importantly, the direct observation of the solid phase by means of Focused Beam Reflectance Measurements (FBRM). In this contribution, we present the kinetic study of an esterification reaction with a preceding dissolution step. Computational fluid dynamics provide information to characterise the solid breakage term within the population balance equation, which also comprises a reactive-diffusive term describing the particle dissolution. Novel complementary methods will be presented that allow an advanced kinetic modelling of reacting solid liquid systems

We acknowledge financial support by the SNF (grant no 200021-113473).

[1] Maeder M., Neuhold Y.-M., *Practical Data Analysis in Chemistry. Elsevier, Amsterdam* **2007**
[2] Puxty G., Fischer, U., Jecklin M., Hungerbühler K., *Chimia,* **2006**, *Vol* 60, 605.

Analytical Chemistry **18**

The role of arginine in chemical cross-linking with NHS esters

Stefanie Mädler, Sabrina Gschwind, and Renato Zenobi

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

N-hydroxy succinimide (NHS) esters are often used as specific and efficient cross-linking agents for the characterization of protein interactions. Apart from acylation of the ε-amino group of lysines and the α-amino group of N-termini, occasional side reactions with hydroxyl side chains have been observed due to catalytic effects. [1] In order to clarify whether arginine (Arg) has an enhancing effect on the acylation of hydroxyl groups by homobifunctional cross-linkers, we carried out systematic experiments with model peptides containing Ser, Thr or Tyr in close proximity to Arg and control samples without Arg. Arg residues were covalently blocked using 2,3-butanedione and phenylboronic acid. [2] Systematic cross-linking experiments were carried out with two homobifunctional cross-linkers. Samples were analyzed by MALDI-TOF-MS (Bruker UltraFlex II) comparing relative reaction yields of intramolecular crosslinks with covalently protected and unprotected Arg. Reactions of Ser, Thr and Tyr occurred predominately as intramolecular linkages connecting the N-termini and the hydroxyl groups. All peptides having high cross-linking yields with unprotected Arg showed a lower reactivity of the hydroxyl groups when Arg was covalently blocked. In control experiments with an Arg-free peptide DPAFNSWG-NH_2, no changes occurred. As catalytic mechanism, the formation of hydrogen bonds between the guanidinium hydrogens and the oxygen of the attacked carbonyl group of the cross-linker should be considered to facilitate the nucleophilic attack of the hydroxyl groups by lowering the activation barrier of the nucleophilic substitution.

[1] S. Madler, C. Bich, D. Touboul, R. Zenobi, *J. Mass Spectrom.* **2009**, *44*, 694.
[2] A. Leitner, W. Lindner, *J. Mass Spectrom.* **2003**, *38*, 891.

Analytical Chemistry **19**

Rapid determination of the gas-phase basicity of MALDI matrices by bracketing approach directly in an electrosonic spray ionization plume

Lukas Fritsche, Konstantin Barylyuk, Roman M. Balabin, Rob Nieckarz, and Renato Zenobi

ETH Zürich, Department of Chemistry and Applied Biosciences Wolfgang-Pauli-Str. 10 8093 Zürich, Switzerland

MALDI is an effective ionization technique for generating ions of large molecules for analysis by mass spectrometry. Although the mechanism of MALDI is still not completely understood, gas-phase proton transfer (PT) from matrix (M) ions to analyte molecules (A) plays an important role in producing ions.

$$[M+H]^+ + A \longrightarrow M + [A+H]^+$$

The efficiency of PT depends strongly on the gas-phase basicities (GB) of the matrix and the analyte.

In the present study we demonstrate a simple method for the rapid determination of the GB of MALDI matrices. Protonated gas-phase ions of several commonly used MALDI matrices were produced by electrosonic spray ionization (ESSI) at ambient conditions, and a set of reference bases with known GB was introduced into the ESSI plume as vapours.

The PT reaction was monitored by the relative signal drop of $[M + H]^+$ in the mass spectrum. The results of GB measurements were compared to theoretical values determined from quantum chemical calculations at the B3LYP level of theory.

[1] D. Touboul, M. C. Jecklin, R. Zenobi, *J Am Soc Mass Spectrom.* **2008**, *19*, 455-466.
[2] R. Knochenmuss, *Analyst.* **2006**, *131*, 966-986

Analytical Chemistry **20**

The softness of different electrospray-based ionization techniques: Analysis of hydrophobically bound supramolecular complexes

Konstantin Baryluk[1], Dan Grünstein[2], and Renato Zenobi[1]

[1]ETH Zurich, Department of Chemistry and Applied Biosciences, Wolfgang-Pauli-Strasse 10, 8093 Zurich, Switzerland
[2]Max Planck Institute of Colloids and Interfaces, Wissenschaftspark Potsdam-Golm, Am Mühlenberg 1 OT Golm, 14476 Potsdam, Germany

The development of soft ionization techniques opened an access to probing large molecules by mass spectrometry. Although the covalent structure of analytes generally remains intact, preserving non-covalent interactions in the gas phase of a mass spectrometer still represents a challenge. Among various types of non-covalent forces hydrophobic interactions are considered to be the weakest and are often totally disrupted upon ionization and ion transfer process.

Herein, we present a comparative study on the softness of several electrospray ionization (ESI)-based techniques for the analysis of supramolecular complexes, which are stabilized exclusively by hydrophobic interactions. Besides the conventional ESI and the nano-ESI, the home-built electrosonic spray ionization (ESSI) and the cold-spray ionization (CSI) sources were used. Mass spectra were recorded with a commercial quadrupole time-of-flight mass spectrometer. The model system consisted of Ruthenium-based core with a controlled number of hydrophobic anamantyl moieties attached, which formed inclusion complexes with cyclodextrin receptors in solution. The stoichiometry of complexes was determined directly from the mass spectrum. The relative stability of fully and partially assembled complexes was studied and compared for all the ESI-based methods used. The influence of ESI parameters on the survival of hydrophobically-bound complexes in the mass spectrometry analysis was systematically explored.

Analytical Chemistry **21**

Asphaltene adsorption onto an iron surface: Near infrared (NIR), Raman, and AFM study

Roman M. Balabin,[1] Rustem Z. Syunyaev,[2] Thomas Schmid,[1] Johannes Stadler,[1] and Renato Zenobi[1]

[1]Department of Chemistry and Applied Biosciences, ETH Zurich, 8093 Zurich, Switzerland
[2]Gubkin Russian State University of Oil and Gas, 119991 Moscow, Russia

A combination of near infrared (NIR) spectroscopy, Raman microscopy, and atomic force microscopy (AFM) was used to analyze the adsorption behavior of asphaltenes [1-5] on an iron (Fe) surface. A Langmuir model was used for analysis of the experimental data [6]. Kinetic and thermodynamic parameters (Gibbs energy of adsorption, adsorption/desorption rate constant, maximal adsorbed mass density) of asphaltenes adsorbed from benzene solution were evaluated: the maximal adsorbed mass density (Γ_{max}) was found to be 4.90±0.07 mg m^{-2}; the adsorption constant (K) was found to be 0.084±0.007 L mg^{-1}; and a value of 34.3±0.2 kJ mol^{-1} was calculated for the Gibbs energy of absorption ($-\Delta G_{ads}$). The structure of the adsorbed layer was analyzed by AFM. Asphaltenes were found to form aggregates on Fe surface with an average size of a few hundred nanometers [6,7]. The conclusions of Sjöblom and co-workers [8] about the non-uniform distribution of petroleum macromolecule on metal surfaces have been confirmed.

[1] R. Z. Syunyaev, R. M. Balabin, *J. Dispersion Science Tech.* **2007**, *28*, 419.
[2] J. G. Speight, *Chemistry and Technology of Petroleum*. CRC; 1999.
[3] R. Z. Syunyaev, *et al.*, *Energy Fuel* **2009**, *23*, 1230.
[4] R. M. Balabin, R. Z. Safieva, *J. Near Infrared Spec.* **2007**, *15*, 343.
[5] O. C. Mullins, E. Y. Sheu, A. Hammami, A. G. Marshall, *Asphaltenes, Heavy Oils, and Petroleomics*. Springer; 2006.
[6] R. M. Balabin, R. Z. Syunyaev. *J. Colloid. Interface Sci.* **2008**, *318*, 167.
[7] R. M. Balabin, *Chem. Phys.* **2008**, *352*, 267.
[8] P. Ekholm, *et al.*, *J. Colloid Interface Sci.* **2002**, *247*, 342.

Analytical Chemistry **22**

Nanoscale Chemical Analysis of Cell Membrane Constituents Using Tip-Enhanced Raman Spectroscopy

Lothar Opilik, Thomas Schmid, Johannes Stadler, Roman M. Balabin, Renato Zenobi

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

Many of the membrane's biological functions have been shown to depend on local environments with specific compositions [1]. Investigation of such membrane domains (e.g. lipid rafts) with nanometer resolution is still a very challenging task, especially when it is done in their native environment.
Tip-enhanced Raman spectroscopy (TERS) is a non-destructive analytical technique capable of yielding vibrational spectra of samples with a lateral resolution below 30 nm [2]. It is essentially an apertureless near-field technique where conventional optics are used to illuminate a metal or metalized scanning probe microscopy (SPM) tip. This tip is brought in close proximity to a sample surface leading to a significant enhancement (several orders of magnitude) of the Raman scattering from the molecules located in the small region under the tip apex. TERS investigations on biological materials can be very challenging due to their non-resonant character. A highly optimized TERS setup is therefore required, especially with respect to tip fabrication.
TERS was successfully performed on supported lipid bilayers (SLBs) with varying complexity. The data was compared to reference spectra from individual membrane components, which were collected using confocal Raman spectroscopy as well as surface-enhanced Raman spectroscopy (SERS). Raster scans on SLBs provided information on the distribution of certain membrane constituents with nanometer spatial resolution.
Our results pave the way for detailed nanoscale chemical analysis of cell membranes in their natural physiological environment, which will give valuable insights into the working mechanisms of a cell.

[1] S. Semrau and T. Schmidt, *Soft Matter* **2009**, *5*, 3174.
[2] B. Pettinger, B. Ren, G. Picardi, R. Schuster and G. Ertl, *J. Raman Spectrosc.* **2005**, *36*, 541.

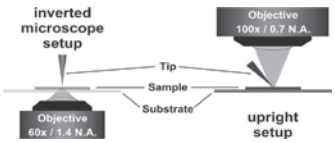
Analytical Chemistry **23**

Tip-Enhanced Raman Spectroscopy for Nanoscale Chemical Analysis of Transparent and Opaque Samples

Thomas Schmid, Johannes Stadler, Lothar Opilik, Roman M. Balabin, Renato Zenobi

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

Biological processes are often strongly affected by nanostructures. For example, nanostructures on the cell surface and in the extracellular space play important roles in bacterial adhesion and biofilm formation. The arrangement of cell membrane constituents affects various biochemical processes. Furthermore, nanoscale structure and composition of modern materials (e.g. thin-film solar cells) significantly influence their macroscale properties.



Tip-enhanced Raman spectroscopy (TERS) is an analytical technique that allows imaging and chemical analysis of different materials with a spatial resolution of 10–50 nm. Our setups for TERS are combinations of confocal Raman microscopy and AFM or STM. Recently, we demonstrated for the first time TERS of opaque samples with the tip and sample surface illuminated perpendicularly from top and the backscattered light collected through the same optics. The TERS effect was observed with both, specially prepared STM and AFM tips in contact with the sample. This overcomes drawbacks of side-illumination schemes usually applied for opaque samples and opens up new fields of application.

The presentation will explain the TERS setups and show applications of TERS and AFM–Raman combinations in the fields of biofilms, lipid layers, and novel solar cell materials.

Analytical Chemistry **24**

Online separation of elements and interferences for LA-ICPMS: electrothermal aerosol heating

Robert Brogioli, Bodo Hattendorf, Joachim Koch, Helmar Wiltsche, Luca Flamigni, Detlef Günther

Laboratory of Inorganic Chemistry, ETH Zurich, Wolfgang-Pauli Strasse10, 8093 Zurich, Switzerland

Laser ablation as sample introduction system for inductively coupled plasma mass spectrometry (ICPMS) has been demonstrated to be a suitable analytical technique for direct trace element analysis and isotope ratio measurements in solid samples. Nevertheless, all components of the ablated sample are introduced into the ICPMS and a selective removal of elements causing spectral or non spectral interferences has not been feasible up to date. This work is based on an initial study of Vaculovic *et* al. [1] and aims at online, selective elements separation from laser generated aerosols by electrothermal heating, hence reducing isobaric interferences and reducing matrix related spectroscopic and non-spectroscopic interferences. For this purpose, the laser generated aerosols of a set of reference materials (NIST 610, MBH B26, BAM M381, BAM M601 and pure Ta) were electrothemally heated within a graphite furnace (HGA600 MS, Perkin Elmer) before introduction into the ICPMS. The temperature dependence of ion signals of major and trace elements, contained in the over mentioned reference materials, was studied. A decrease of ion signal intensity was detected for individual elements at characteristic temperatures depending on the reference material. The signal intensity for volatile elements (Ag, Cd) was reduced down to 0.1 % when heating the laser generated aerosols of NIST 610. For the brass sample the signal of Zn and Cu was reduced down to 0.01 %. In order to study the effect of thermal treatment on the aerosol particle size after electrothermal heating, the aerosols were directly visualized by mean of laser light scattering showing a temperature dependent change of the particle size.

[1] Vaculovic T., *Can. J. Anal. Sci. Spectros.* **2004**, *49*, 353.

Analytical Chemistry **25**

Effect of carbon introduced via an Electrothermal Vaporization unit on sensitivity in LA-ICPMS

Bahir Duraki, Robert Brogioli, Bodo Hattendorf, Detlef Günther

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

The controlled addition of small amounts (µL/min) of organic solvents (e.g. methanol or acetone) has been demonstrated to effectively enhance sensitivity for solution based inductively coupled plasma mass spectrometry (ICPMS), especially for elements with high first ion potential.[1] For dry plasma conditions, the addition of methane, hydrogen and nitrogen to the carrier gas of laser generated aerosols has been studied by Guillong *et al*[2]. and a sensitivity enhancement has been reported to be 5-7 fold for elements with high ionization energies as As, Pt, P and Au when adding hydrogen. The addition of methane has been shown to have a similar effect but less pronounced whereas the addition of nitrogen has been shown not to enhance the figure of merit.
In this study an alternative source of carbon for the enhancement of sensitivity in LA-ICPMS was evaluated and compared to the conventional addition of gaseous organics. For this purpose an ETV unit was connected to an ICPMS system in parallel with a laser ablation system. Heating up the graphite furnace to temperatures above 1000 °C allowed the controlled introduction of carbon into the plasma parallel to the laser generated aerosol. The signal behavior of measured isotopes was evaluated for furnace temperatures ranging from 1000 °C to 2650 °C. For elements with relatively high first ionization potential like As, Se, P and Te a 1.66 – 2.33 fold increase in sensitivity increase was obtained.

[1] Z. Hu, S. Hu, S. Gao, Y. Liu, S. Lin, *Spectrochim. Acta, Part B.* **2004**, *59*, 1463-1470.
[2] M. Guillong, C.A. Heinrich, *J. Anal. At. Spectrom.* **2007**, 22, 1488-1494.

Analytical Chemistry **26**

KINONE
A new software for the kinetic modelling of spectroscopic, calorimetric and concentration data

Julien Billeter, Yorck-Michael Neuhold, Konrad Hungerbühler

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

On this poster, we present the new software *Kinone* dedicated to the kinetic modelling of spectroscopic, calorimetric and concentration data in homogeneous liquid phase. Its capabilities will be demonstrated using some selected kinetic problems.

Kinone is organised in two modules connected to a user-friendly interface with comprehensive graphical output.

The first module is a model-based (hard-modelling) unit that can be used in Simulation or Fitting mode and under various experimental conditions such as batch and semi-batch (inlets/outlets), as well as isothermal and non-isothermal conditions. This module can simultaneously handle several kinetic models. This makes *Kinone* particularly suitable for automatic model screening. In Fitting mode, multiple objective functions as well as various kinetic and experimental parameters can be fitted by a non-linear gradient-based method. Pure component spectra (absorptivities) and reaction enthalpies are calculated without any calibration. If some of these spectra and enthalpies are independently known, they can also be incorporated into the analysis. *Kinone* also allows the simultaneous local or global analysis of multiple data sets by defining appropriate groups.

The second module is a model-free (soft-modelling) unit that helps identifying the number of reactive species and elaborating plausible kinetic model candidates. It relies on methods such as PCA, EFA and MCR-ALS.

Kinone will be available for download free of charge as a *Matlab* package or as an executable file from www.sust-chem.ethz.ch/downloads

Analytical Chemistry **27**

A Laser Ablation millisecond-pulsed Glow Discharge Time-Of Flight Mass Spectrometer (LA-GD-TOFMS) for Elemental and Molecular Analysis

Mohamed Tarik[1], Detlef Günther[1]

[1] ETH Zurich, D-CHAB, Laboratory of inorganic chemistry, Wolfgang-Pauli-Str. 10, 8093 Zurich, Switzerland

Over the last few years, several groups have examined the possibility of analyzing solids using laser ablation (LA) based Glow Discharge (GD) setups in combination with atomic emission detection. In this study, an in-house built Laser Ablation ms-pulsed Glow Discharge Time-Of-Flight Mass Spectrometer (LA-GD-TOFMS)[1] was used for the analysis of prepared organic (benzoic acid and vanillic acid), polymeric (PTFE and PVC) samples and a commercially available pharmaceutical tablet . The benzoic and vanillic acid samples were prepared both without and with (200 ppm) doping elements (Al, Cu and Pb). Analyses of these compounds were accomplished by introducing the laser ablated material into two different temporal regimes of the pulsed GD (plateau and afterglow).

The mass spectra of both acids recorded in the plateau contained both elemental and structural information. Through electron impact ionization, the plateau provides therefore an intermediate effective ionization potential. In the afterglow, the acquired spectra show the presence of the intact parent molecule M^+ of the analyte and the protonated molecule $[M+H]^+$. Penning ionization is assumed to be the dominant mechanism involved in the generation of parental molecular ions giving the GD plasma a “soft ionization character” in the afterglow. For both polymers, the low-mass and medium-mass fragments were recorded during the plateau and the afterglow, respectively. For the doped samples, all the doped elements were detected with a LOD of about 20 ppm. The two main compounds (caffeine and paracetamol) in the pharmaceutical tablet were successfully identified.

[1] M. Tarik, G. Lotito, J. A. Whitby, J. Koch, K. Fuhrer, M. Gonin, J. D. Gunther, *Spectrochimica Acta Part B-Atomic Spectroscopy*, 2009, **64**, 262.

Analytical Chemistry **28**

Influence of Oxygen on the Formation of Gas Species in LA-ICPMS

Steffen Allner, Daniel A. Frick, Detlef Günther

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

Laser ablation-inductively coupled plasma mass spectrometry (LA-ICPMS) is now routinely applied for the analysis of human remains (hair, tissue etc.), cellulose and polymers. These "new" type of samples are organic materials. Therefore, matrix matched standards containing a wide variety of elements for quantification of trace elements are required.

Due to the matrix, carbon and sulphur have been frequently used as internal standards for such applications [1]. Unfortunately, the representativeness of the ablation of an organic matrix as well as the influence of the composition of the matrix on the aerosol composition and the transport efficiency are currently unknown. However, the effect of small amounts of O_2 on laser-induced fractionation of metals has been demonstrated by Kosler *et al*. [2]. Therefore the behaviour of oxophilic non-metal elements such as carbon, sulphur and phosphorous in LA-ICPMS was investigated. The oxides of these elements are gases (CO_2, CO, SO_2) and it can be assumed that gas phases behave differently during transport and ionization than particles. With the recently introduced gas exchange device by Kovacs *et al.* [3] such gas phases can be separated without changing the composition of the particles and without changing the ICP conditions.

We will present evidence on the formation of gas species during laser ablation with special focus on the influence of O_2 addition to the carrier gas flow used for the aerosol transport.

[1] H. Sela, Z. Karpas, M. Zoriy, C. Pickhardt and J. S. Becker, *Int. J. Mass Spectrom.*, **2007**, *261*, 199-207.
[2] J. Kosler, H. P. Longerich and M. N. Tubrett, *Anal. Bioanal. Chem.*, **2002**, *374*, 251-254.
[3] R. Kovacs, K. Nishiguchi, K. Utani and D. Günther, *J. Anal. At. Spectrom.*, **2010**, *25*, 142-147.

Analytical Chemistry **29**

"Mobile" laser ablation ICPMS – A proof of concept

Reto Glaus, Detlef Günther

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

Laser ablation inductively coupled plasma mass spectroscopy (LA-ICPMS) allows quantification of main, minor and trace elements in solid samples without any sample preparation step (e.g. digestion). A mobile device would be of interest, in order to enable LA-ICPMS to samples beyond the lab, e.g. for archeological samples or museum pieces. However, whereas small portable laser systems are available, the ICPMS is limited to the use in the lab, due to the need for gas-, electricity- and cool water-supply. In this work the capabilities for trace element analysis of solid samples by demobilization of the laser generated aerosols and following remobilization into the ICPMS were investigated.

The sampling was performed by laser ablation of the target and demobilization of the generated aerosols on nuclepore membrane filters. After this offline LA step, the filters were reablated by scanning LA and quantified in the ICPMS against an external standard. This offline-LA LA-ICPMS method was applied to the quantification of trace elements in glass, metal and sulfide samples. The setup allowed quantifications with similar performance as with direct LA, by using a 213nm Nd:YAG laser system (5th harmonic wavelength) for the first and second LA step. However, higher limits of detection has to be taken in account for most of the elements (few ppm instead of sub-ppm range with direct LA-ICPMS), mainly due to background signals from the filters.

The concept of mobile laser ablation followed by remobilization into the ICPMS was successfully tested. Quantification was applied to metals and non-metals without serious disadvantages compared to direct LA-ICPMS. In a next step a mobile laser device has to be assembled and its performance has to be evaluated for the trace element quantification on "real" samples.

Analytical Chemistry **30**

Analyse of Rosin by Gas Chromatography Mass Spectrometry from Basketry immersed for 2500 years.

Armelle Vallat[1], Géraldine Voumard[2]

[1]University of Neuchâtel, Ave de Bellevaux 51, CH-2000 Neuchâtel, Switzerland
[2]Laténium, Archeology museum, Espace P. Vouga, CH-2068 Hauterive, Switzerland

The basketry was discovered on a shipwreck lying on the Sicilian coast, facing the *emporion* of the ancient Greek city of Gela. This almost complete basketry is in a surprisingly good conservation state, giving it was immersed for 2500 years. The gas chromatography coupled to mass spectrometry with derivatisation reaction has been used to establish that the basketry is made out of rosin. The rosin [1] is the least volatile fraction of the distillation of turpentine extracted from pine by resin tapping and it constituted at more 70% of resin acids, formula $C_{20}H_{30}O_2$.
The rate of autoxidation of resin acids has been reported using an "index for the degree of oxidation" (IDOX) [2], which allows a more quantitative measure of the degree of ageing in rosin of basketry.

[1] A.J.D. Silvestre, A. Gandini, in 'Monomers, polymers and composites from renewable resources', Ed. M. Belgacem, A. Gandini, Elsevier Ltd., **2008**, p. 67.
[2] K.J. van Den Berg, J.J. Boon, I. Pastorova, L.F.M. Spetter, *J. Mass Spectrom.* **2000**, *35*, 512.

Analytical Chemistry **31**

Lysophosphatidylcholine derivatives from goji berries (*Lycium barbarum*): detection and isolation by MS-coupled preparative HPLC

Regina Wenger, Orlando Fertig, Matthias Hamburger, Olivier Potterat

Division of Pharmaceutical Biology, University of Basel, Klingelbergstrasse 50, CH-4056 Basel

Goji (*Lycium barbarum*, Solanaceae) berries and juice are increasingly popular as health food products and praised in advertisements and in the media for well-being and as an anti-aging remedy [1]. While the fruit itself is devoid of toxicity there are concerns about the quality of goji products with respect to pesticide contamination or possible adulteration.

As part of our investigations on goji, we detected in HPLC chromatograms of extracts a group of late-eluting compounds lacking UV absorption. An approach based on MS-coupled preparative HPLC was used for the isolation of these compounds. The experimental setup consisted of a HPLC-MS instrument equipped with an adjustable flow splitter and an additional pump delivering a make-up flow. Separations were performed on a semi-preparative RP-18 HPLC column (10 x 150 mm, i.d.) and afforded eight compounds in sub-milligram to milligram amounts. They were identified as lysophopsphatidylcholine derivatives with fatty acid residues of variable length and degree of unsaturation, by a combination of spectroscopic and chemical methods including ESI-MS, 1D- and 2D-NMR, and GC-MS analysis of the acyl residues after methanolysis. Interestingly, a mixture of phosphatidylcholine and lysophosphatidylcholine derivatives with a related fatty acid composition has been detected in jojoba seed meal [2]. On the other hand, such metabolites have not been reported so far in goji berries. These compounds may be useful as chromatographic markers for the analysis of goji products. At the same time they may give new hints with respect to the biological properties of goji berries and products.

[1] Potterat, O. *Planta Med.* **2010**, *76*, 7.
[2] Leon F, Van Boven M, De Witte P, Busson R, Cokelaere M *J. Agric. Food Chem*. **2004**, *52*, 1207.

Analytical Chemistry **32**

Rapid separation of plant extracts by Flash chromatography on cartridges – Principles for the selection of chromatographic conditions and comparison with medium pressure liquid chromatography

Olivier Potterat[1], Petra Weber[1], Nina Schafroth[2], Matthias Hamburger[1]

[1]Division of Pharmaceutical Biology, University of Basel, Klingelbergstrasse 50, CH-4056 Basel
[2]Büchi Labortechnik AG, CH-9230 Flawil

Flash chromatography on cartridges has become increasingly popular for the rapid purification of compounds, mainly of synthetic origin. In contrast, its application for natural product isolation is poorly documented, and easy-to-use procedures for optimization of the separation conditions are lacking. Using Sepacore® cartridges (*Büchi Labortechnik*), we have established empirical rules for the selection of chromatographic conditions with an emphasis on gradient mode. Reversed phase HPLC separations can be transposed by increasing the gradient time by a factor 2-4. For normal phase separations, solvent compositions resulting in R_f values of 0.15 - 0.2 on TLC for the most lipophilic and the most hydrophilic constituents, respectively, should be selected as gradient endpoints. We applied these rules to the separation of complex plant extracts, with *Curcuma xanthorrhiza*, *Piper nigrum* and *Salvia milthiorrhiza* as examples of medicinal and commercial importance. The performance of the cartridges was compared to that of classical MPLC (medium pressure liquid chromatography) glass columns. Sepacore cartridges enabled a good separation of compounds with a broad range of polarity, as typically found in plant extracts. The chromatographic resolution remained, however, lower than that achieved by MPLC on columns packed with material of smaller particle size. For poorly soluble extracts, solid introduction gave better results than liquid injection. Despite lower resolution as compared to MPLC, pre-packed cartridges are an attractive alternative for the purification of extracts and crude fractions due to their ease of use and speed of separation.

Analytical Chemistry **33**

High-Resolution Tandem Mass Spectrometry

of Modified Oligoribonucleotides

Adrien Nyakas, Silvan Stucki and Stefan Schürch[1]

[1]University of Bern, Department of Chemistry and Biochemistry, Freiestrasse 3, 3012 Bern

Shortly after the role of genes and the corresponding translational machinery was elucidated, a lot of effort was undertaken, to manipulate this complex biochemical process. Several approaches have been developed, known as e.g. antisense, antigene, or RNA interference strategies. The goal is to influence the translation process by inactivation of a certain nucleic acid sequence using short complementary single strands. Since native oligonucleotides (ONs) are very prone to decomposition, various modifications were implemented to prevent enzymatic degradation in a cellular environment. Chemical alterations though, often render classic ways of sequencing, such as the Sanger method, impossible. A powerful tool to overcome this limitation is high-resolution tandem mass spectrometry (MS/MS). Complete sequence information can be obtained very fast, consuming only little amounts of sample. However, the fragmentation pattern of modified and native nucleic acids differs, depending on the type and position of the alteration. So far, rather little is known about the inherent dissociation pathway of ONs containing chemical variations, so that *de novo* sequencing turns out to be difficult.

To gain deeper knowledge about preferred cleavage sites of highly modified nucleic acids under CID conditions, single stranded ONs of identical base composition, but differently located modifications, were investigated on a LTQ Orbitrap XL (Thermo Fisher Scientific, Bremen, Germany) equipped with a nano electrospray ionization source. The obtained high-resolution MS/MS spectra revealed a considerably different fragmentation behavior if the modification was shifted within the sequence. Based on the recorded data, gas-phase dissociation mechanisms for the investigated modified ONs are proposed in order to assign the observed fragments.

Analytical Chemistry **34**

DNA Quadruplexes: A new Challenge for Mass Spectrometry

Silvan Stucki, Adrien Nyakas, and Stefan Schürch[1]

[1] Department of Chemistry and Biochemistry, University of Bern, Bern, Switzerland

Since the biological formation of nucleic acid secondary structures turned out to be of significant importance for many organisms, the development of adequate analytical methods has been promoted. Among the analytical techniques available nowadays, high resolution electrospray ionization mass spectrometry represents one of the most sophisticated tools for accurate and rapid characterization of nucleic acid structures, including duplex and quadruplex DNA.

DNA quadruplexes attracted notice during the past decade and the list of potential tasks these guanine-rich tetraplex structures can fulfill is growing rapidly. Sequence analysis revealed the presence of quadruplex repeats in telomeric regions of many organisms e.g. yeasts, ciliate protozoa, and human. They are believed to maintain regulatory functions of cell cycle control. Especially in relation with malicious cancer cells, telomere maintenance and quadruplex formation seem to play crucial roles. However, many further functions are predicted for quadruplex structures, such as participation in gene transcription, regulation, and chromosome reorganization.

Increased activity in this field of research demands precise and fast analytical tools for structural elucidation of noncovalently bound quadruplex structures. In the present study, various quadruplexes were investigated by full scan and tandem mass spectrometric experiments. Data were acquired on a LTQ Orbitrap XL instrument (Thermo Fisher Scientific, Bremen, D) equipped with a nano-electrospray ion source. Additionally, the stability and fragmentation pathways of the complexes between DNA quadruplexes and potent organometallic ligands were investigated.

Analytical Chemistry **35**

Atmospheric pressure sampling for laser ablation based nanoscale imaging mass spectrometry: ions or neutrals?

Liang Zhu[1], Johannes Stadler[1], Thomas A. Schmitz[1], and Renato Zenobi[1]

[1]Department of Chemistry and Applied Biosciences, ETH Zürich, 8093 Zürich, Switzerland

Although the ratio of neutrals-to-ions in a typical laser ablation event was reported to be of the order of 1000 or greater, most imaging mass spectrometry (IMS) studies collect the minor ionic component instead of the abundant neutrals for subsequent mass analysis. In this report, we present a fundamentally different strategy, sampling neutrals from atmospheric pressure laser ablation into the vacuum of the mass spectrometer, followed by post-ionization, and compare its overall efficiency (transfer efficiency + ionization efficiency) with that of ion sampling. The products from single ablation events creating a crater of ~1 μm in diameter were deposited on a collection plate placed on the vacuum side of the sampling capillary. The sample surface and the collection plate were carefully examined both prior to and after laser ablations with scanning electron microscopy (SEM) and scanning probe microscopy (SPM). Volumetric measurements gave a rough estimate of the overall sampling efficiency of ~10^{-4}. It was found that using a proper collection geometry, ablated neutral molecules can be efficiently directed to the inlet of the sampling capillary: several percentage are available for further MS analysis. It was also revealed that the fraction of the ablated mass in the form of particle was not sampled into the vacuum, but was deposited between the ablation site and the capillary inlet. By comparing our results with other potential IMS techniques using ion sampling, we conclude that the overall sampling efficiency is similar. Although the effective sampling volume within the ablation plume is enlarged by the electric field in the case of ion collection, several advantages provided by the neutral sampling approach, such as the amount of analyte available for collection, the potential for improving the ionization efficiency, and the elimination of elaborate sample pre-treatments, can compensate for the absence of an increased effective sampling volume.

Analytical Chemistry **36**

On the Mechanism of Extractive Electrospray Ionization

Rui Wang[1], Wai Siang Law[1], Huan Wen Chen[2], Renato Zenobi[1]

[1]Department of Chemistry and Applied Biosciences, ETH Zurich, CH-8093, Switzerland

[2]East China Institute of Technology/Department of Applied Chemistry, Fuzhou, 344000, China

Extractive electrospray ionization (EESI)-mass spectrometry (MS) was first introduced by Chen et al.[1] to analyze samples with complex matrices. In the EESI process, the analytes are first aerosolized/nebulized and then ionized by collision with charged droplets generated by an ESI spray formed from pure solvent. As a consequence, no sample pretreatment is needed. Despite its importance, there is still a lack of detailed studies on the ionization mechanism of EESI. In this work, we studied the mechanism of EESI using in-plume laser-induced fluorescence (LIF) and mass spectrometry. In the LIF study, rhodamine 6G (R6G) was used to figure out whether a liquid-phase or a gas-phase interaction predominates in the EESI process. No fluorescence from gas-phase R6G ions was observed even 30 mm away from the origin of the EESI plume. This implies that the interaction between the charged ESI droplets and neutral sample is mainly a liquid-phase interaction. Furthermore, we investigated how the analyte solubility influences the ion signals in EESI-MS measurements using nile red in a methanol/chloroform mixture. The ion intensity of protonated nile red was found to be enhanced with increasing chloroform fraction in the primary ESI spray. Since nile red has a higher solubility in chloroform than in methanol, we can conclude that (1) nile red molecules prefer to be extracted from the neutral sample droplets into the charged ESI droplets, implying the obvious influence of analyte solubility on the ion intensity of the analyte. (2) a selective extraction takes place during the collision of the charged ESI droplets and the neutral sample droplets in the EESI process.

[1] Chen, H. W.; Venter, A.; Cooks, R. G., *Chem. Comm.* **2006**, *19*, 2042.

Analytical Chemistry **37**

Investigating the behavior of Cytocrome C in Electrospray and Electrosonic Spray Ionization Plumes

Pitt Allmendinger[1], Rui Wang[1], Renato Zenobi[1]

[1]Department of Chemistry and Applied Biosciences, ETH Zurich, CH-8093, Switzerland

Electrospray ionization (ESI) and electrosonic spray ionization (ESSI), as soft ionization methods [1], are widely used techniques to investigate biological molecules. However, it is still not fully understood how biomolecules, especially proteins, are influenced by the ionization process. Noting that the charge state and charge state distribution of proteins often correlate with to the degree of unfolding during the ionization process [2], we studied the charge state and charge state distribution of cytochrome C at various positions in both ESI and ESSI plumes using mass spectrometry (MS). In our measurements, a clear dependence of the charge state and the charge state distribution was observed along both the axial and radial directions inside the plume. In the axial direction, the charge state shifted to higher values and the charge state distribution became broader when the emitter of the electrospray capillary was moved closer to the entrance of the MS instrument, suggesting that the desolvation is less complete at a closer position. Along the radial direction in the plume, more ion signals of lower charge states were obtained further away from the center of the plume, which implies that desolvation is more complete at the periphery of the plume.

[1] Z. Takáts, J.M. Wiseman, B. Gologan, R.G. Cooks, *Anal. Chem.* **2004**, *76*, 4050.

[2] M. F. Jarrold, *Acc. Chem. Res.* **1999**, *32*, 360.

Analytical Chemistry **38**

Laser Spectroscopy of Trapped Green Fluorescence Protein Ions

Frankevich V., Chingin K., Nieckarz R., Zenobi R.

ETH Zuirich, Deprtment of Chemistry and Applied Biosciences HCI, 8093, Zurich

The combination of laser-induced fluorescence (LIF) with mass spectrometry opens up new possibilities both for the detection purposes and for structural studies of trapped biomolecular ions in the gas phase. Study of large molecular systems in the gas phase has experienced an enormous boost by the introduction of soft ionization methods for mass spectrometry (MS), in particular electrospray ionization (ESI) and matrix-assisted laser desorption/ionization (MALDI). These are capable of producing intact ions from high molecular weight, completely nonvolatile species.

We describe the discovery that completely unsolvated ions are present in abundance inside an ESI plume already at ambient conditions. This was done by comparing fluorescence spectra obtained in different regions of the ESI plume with those from isolated ions trapped in FTICR. Thus, we were able to obtain fluorescence spectra of gas-phase Green Fluorescent Protein (GFP), chlorophyll b, and polypeptides labeled with a fluorescent tag. Also, our data suggest that the ion evaporation model (IEM) is the main driving force responsible for ion formation in ESI, at least for small molecules. The optical propeties of GFP ions were probed when transferred from solution into the gas phase by ESI-MS: in solution, in the ESI plume and when trapped inside the ICR measuring cell. Our data strongly suggest that the protein ions can be desolvated directly inside the ESI/ESSI plume at ambient conditions, while maintaining their near-native tetriary structure responsible for the native fluorescence of GFP. Data obtained in FTICR-MS provides some indirect support that gas-phase GFP ions can preserve their intact shape for longer time scales, idicating a high stability of the native conformation position.

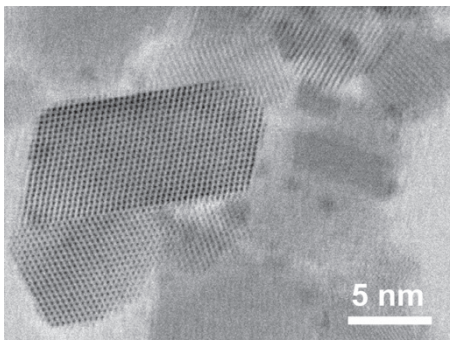
Analytical Chemistry **39**

Aberration-corrected STEM for catalyst characterization

F. Krumeich[1], E. Müller[2] , R. A. Wepf[2],and R. Nesper[1]

[1]Laboratory of Inorganic Chemistry, ETH Zürich, CH-8093 Zürich
[2] EMEZ, ETH Zürich, CH-8093 Zürich

Resolutions in the sub-Ångström range can be achieved in a scanning transmission electron microscope (STEM) that is equipped with a corrector system removing the spherical aberration of the probe-forming lens. Bright field (BF), annular dark field (ADF) and secondary electron detectors are attached to this microscope, a Hitachi HD-2700CS, making it a versatile tool for materials characterization [1,2]. Here, we demonstrate its potential for the detection of metal nanoparticles supported on oxide substrates.



BF-STEM image of Pt nanoparticles (diameter ca. 1 nm) dispersed on cerium oxide.

[1] H. Inada, L. Wu, J. Wall, D. Su, Y. Zhu *J. Electron Microsc.* **2009**, *58*, 111.

[2] Y. Zhu, H. Inada, K. Nakamura, J. Wall *Nature Mater.* **2009**, *8*, 808.

Analytical Chemistry **40**

Tracking Lithiation and Alkylation/Acylation of Substituted Acetanilides via In-Situ ATR Spectroscopy, Calorimetry and Endoscopy

Tamas A. Godany, Yorck-Michael Neuhold, Konrad Hungerbühler

ETH Zurich, Institute for Chemical and Bioengineering, Wolfgang-Pauli-Str.10, 8093 Zurich, Switzerland

Substituted acetanilides are important precursors in pharmaceutical industry. Functionalization of the aromatic ring or the amide side chain is often achieved by sequential steps of lithiation, alkylation/acylation and hydrolysis [1]. The dynamic description and understanding of the course of such multi-step reactions is of the utmost importance in industry during early process development but also for online optimization at production scale.

In recent years, we have developed high-performance reaction calorimeters [2] with in-situ monitoring via ATR-IR and UV-vis spectroscopy, alongside with endoscopic visualization. The latest reactor generation is particularly suited for low-temperature processes such as lithiation reactions.

In this context, we investigated the reaction of some acetanilide derivatives. We show how in-situ analysis allows to optimize the dosing schedule to avoid side reactions (e.g. the recombination of lithiation and alkylation/acylation agents), to follow heterogeneous steps (e.g. precipitation/dissolution of intermediates), and to detect endpoints of the reaction steps.

We gratefully acknowledge financial support from Lonza AG, Visp.

[1] Rappoport Z. and Marek I. *The chemistry of organolithium compounds*. Wiley, Chichester **2004**.

[2]Puxty G., Fischer U., Jecklin M., Hungerbühler K. *Chimia* **2006**, 60, 605.

Analytical Chemistry **41**

LC-MS/MS quantification of bile acids in patients with deficiencies in reverse cholesterol transport

Carine Steiner, Katharina Rentsch, Arnold von Eckardstein

Institute for Clinical Chemistry, University Hospital Zurich, Rämistrasse 100, 8091 Zurich, Switzerland

Introduction: The aim of this study was to investigate bile acid concentrations in patients with mutations in different genes involved in reverse cholesterol transport (RCT). This mechanism serves to transport cholesterol from peripheral tissues back to the liver. Cholesterol, a poorly soluble membrane lipid is eliminated from the human body by conversion into various water-soluble, amphipathic bile acids, which are then secreted into the small intestine. These bile acids are found in the systemic circulation as a consequence of efficient reabsorption from the small intestine. The effects of mutations in RCT genes on cholesterol levels are well understood, however their impact on bile acid levels remains largely unknown.

Methods: The 15 major human bile acids were quantified using liquid chromatography coupled to mass spectrometry (LC-MS/MS). Samples consisted of 100µl serum from controls (n = 31) and from patients carrying mutations which affect the following proteins: ATP binding cassette transporter A1 (ABCA1) (n = 9), lecithin-cholesterol acyltransferase (LCAT) (n = 15), cholesteryl ester transfer protein (CETP) (n = 3), apolipoprotein A-I (apoA-I) (n = 3) and scavenger receptor BI (SR-BI) (n = 8).

Results: The most interesting and surprising results of this study showed that serum concentrations of bile acids conjugated with glycine or taurine (in contrast to unconjugated bile acids) were increased in patients carrying mutations in the SR-BI gene. An identical tendency was observed for primary bile acids, which comprise unchanged forms of bile acids synthesized in the liver (in contrast to secondary bile acids which have been modified by intestinal bacteria). In addition, the same bile acids (primary and conjugated) were also increased in patients carrying mutations in the apoA-I gene.

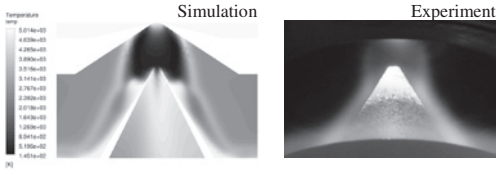
Analytical Chemistry **42**

The way to a new ICPMS interface - CFD Investigations on the Plasma expansion in a ICPMS interface.

Rolf Dietiker, Tatiana Egorova, Bodo Hattendorf, Detlef Günther

ETH Zurich, D-CHAB, Laboratory of Inorganic Chemistry, Wolfgang Pauli Str. 10, 8093 Zurich, Switzerland

For trace elemental analysis inductively coupled plasma mass spectrometry (ICPMS) is a well established technique with high analyte sensitivity. Nevertheless ICPMS still suffers from a detection efficiency of less than 10^{-4}[1] mainly caused by the non ideal vacuum interface transferring the atmospheric pressure plasma into an ion beam at low pressures. A new interface type with the goal of higher transmission of the analyte ion is investigated in our group. For a rational interface design a deeper understanding of the flow patterns of a hot plasma during its expansion into the first vacuum stage of the mass spectrometer interface and its dependencies on geometrical guides as well as on applied extraction voltages is important. Computational fluid dynamics (CFD) calculations are applied for the numerical simulation of supersonic expansions in many fields to model the evolution of flow patterns applying different influencing parameters helping to reduce experimental expenses substantially.



Numerical simulations using Ansys CFX 12.1 [2] are compared to experimental results carried out in a in house built modular ICPMS interface on a traditional ICPMS interface and other geometrical setups.

[1] S.Al Moussalami, W. Chen, B.A. Collings, D.J. Douglas, *Rev. Sci. Instrum.,* **2002**, *73*, 884.
[2] ANSYS CFX 12.1, Ansys Inc. www.ansys.com

Analytical Chemistry **43**

Dissipation of the plasma beam inside the ICPMS interface.

Tatiana Egorova, Rolf Dietiker, Bodo Hattendorf, Detlef Günther

ETH Zurich, D-CHAB, Laboratory of Inorganic Chemistry, Wolfgang Pauli Str. 10, 8093 Zurich, Switzerland

Inductively coupled plasma mass spectrometry (ICPMS) is an established technique for trace elemental analysis. Despite its high analyte sensitivity, detection efficiency of current ICPMS is still below 10^{-4} [1]. This is mainly due to the vacuum interface required to transform the atmospheric pressure plasma into an ion beam for MS analysis.
To improve ion transmission a new interface based on configuration without skimmer with an electrodynamic ion funnel was suggested [2]. First experiments with the ion funnel placed directly within the initial expansion stage of the plasma suffered from the high ion currents causing a breakdown of the pseudo-potential inside the funnel. Therefore an additional interface in front of the ion funnel is currently under investigation.
In order to understand the plasma expansion, the development of the jet along and perpendicular to the axis of the beam was studied in detail by optical and electrical measurements. The dissipation of the jet varies for different pressure stages and depends on a pressure gradient. The total ion current detected at different positions downstream the entrance aperture does not vary significantly, while replacing a fraction of the argon carrier gas with helium led to increased total current. The axial position of the mach disk is sensitive to the pressure gradient but does not vary significantly with plasma composition or electric fields applied in the expansion region.

[1] S.Al Moussalami, W. Chen, B.A. Collings, D.J. Douglas, *Rev. Sci. Instrum.,* **2002**, *73*, 884.
[2] Kim, Tolmachev, *Anal. Chem.,* **2000**, *72*, 2247-2255

Analytical Chemistry **44**

On-line detection of semivolatile and nonvolatile compounds in breath aerosols

Christian Berchtold[1], Renato Zenobi[1]

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

Breath contains aerosols which carry semi- and nonvolatile compounds. Semi volatiles are usually analyzed by absorption techniques such as SPME GC-MS. These methods are offline and do not provide the possibility to monitor compounds in breath in real time[1] Currently, most of the MS methods that enable online monitoring detect volatile compounds only. A major problem with detecting low volatility compounds in breath is their low concentration (ppbv to pptv levels)[2]. A LOD in the range of low ppbv would be highly desirable for a useful application, although at the moment no acceptable technology that reaches this benchmark exists. The detection of semi- and nonvolatile compounds was investigated, specifically narcotics, which might be detected in breath. The LODs of a few compounds analyzed on different mass spectrometers by ESI, extractive ESI[3] and APCI were compared. We compared a Q-TOF, commercial ion traps (LCQ, LTQ) as well as a prototype of a portable ion trap (mini 10.5) [4] with respect to their sensitivity and scan speed. Our investigations show that the sensitivity issue is not strongly dependent on the massanalyzer system. The ionization efficiency, as well as the transport efficiency of the neutral and ionized samples, are the crucial steps in this kind of analyses. However, if the sample is completely evaporated and transported well into the gas phase, APCI based ionization technologies seem to be the most promising ionizing system for an on-line monitoring of breath aerosols.

[1] T. Ligor, *Crit Rev Anal Chem* **2009**, *39*, 2.
[2] F. M. Benoit, W. R. Davidson, A. M. Lovett, S. Nacson, A. Ngo, *Anal. Chem.* **1983**, *55*, 805.
[3] J. H. Ding, S. P. Yang, D. P. Liang, H. W. Chen, Z. Z. Wu, L. L. Zhang, Y. L. Ren, *Analyst* **2009**, *134*, 2040.
[4] Z. Ouyang, R. J. Noll, R. G. Cooks, *Anal Chem* **2009**, *81*, 2421.

Analytical Chemistry **45**

Robust capillary electrophoresis–mass spectrometry interface using a miniature flowing atmospheric-pressure afterglow ion source

Stefan Schmid[1], Pawel L. Urban[1], Matthias C. Jecklin[1], Andrea Amantonico[1] and Renato Zenobi[1]

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

The analysis of small molecules in complex samples is challenging. Mass spectrometry (MS) often needs to be hyphenated with an on-line separation technique such as capillary electrophoresis in order to cope with such matrix-rich samples. The most common CE-MS interface is based on the electro spray ionization process. While the sheath-flow design provides a relatively stable means of CE-MS coupling, a disadvantage of this interface is the inherent dilution of the CE effluent. On the other hand, these sheathless interfaces can offer superior sensitivity since there is no dilution effect. Here we present a sheathless coupling interface for capillary electrophoresis with MS using a miniaturized version of the flowing atmospheric pressure afterglow (miniFAPA) ion source.

The main aim of this study was to downscale the flowing atmospheric pressure afterglow (FAPA) ion source and use it for sheathless coupling of CE with mass spectrometry. The miniFAPA ion source was redesigned from our previous work (1), because the PTFE-body of the conventional FAPA source was quite bulky (cylinder with a 45-mm diameter). This would cause problems with alignment, especially when elements such as a microscale CE capillary had to be installed. The capillary end was painted with a conductive paint ("Leistislber 200") which adheres to the polyimide coating of the fused silica capillaries used. The resulting layer is uniform, resistant to friction during assembly of the interface and to most chemicals used in CE.

[1] Jecklin, M.C.; Gamez, G.; Touboul, D.; Zenobi, *R. Rapid Commun. Mass Spectrom.* **2008**, *22*, 2791-2798.

Analytical Chemistry **46**

Mass fractionation study of Copper and Zinc depending on ICP instrumental settings in multi collector-ICPMS measurements

Ladina Dorta[1], Gisela Fontaine[1], Bodo Hattendorf[1], Detlef Guenther[1]

[1] ETH Zürich, Laboratory for inorganic Chemistry, Zurich, Switzerland

Isotope ratio measurements using multi collector inductively coupled plasma mass spectrometry (MC-ICPMS) are affected by significant mass bias effects. Thus, to ensure accurate measurements, the measured intensity ratios need to be corrected for the instrumental bias using an isotope pair with known abundance ratio. In such a case, empirically derived relationships of the mass dependent isotope transmission are employed. Ideally the isotope pair used for correction should be from the same element to minimize chemical effects. For many isotope systems, however, an isotope pair with accurately known abundance ratio is not available.

This study investigates the potential use of inter-element corrections of the mass bias observed in MC-ICPMS as proposed by the literature[1,2]. Zn isotopes are used to monitor the mass bias of a NU Plasma HR MC-ICPMS instrument (NU Instruments, Wrexham, UK) and to correct for variations of the measured Cu intensity ratios.

The influence of instrumental operating conditions as well as sample composition, which have an effect on the respective mass fractionation of the individual elements, was studied for Cu, Zn and brass reference samples with different Cu/Zn mass fractions.

The samples were analyzed by laser ablation for sample introduction and, after digestion using solution nebulisation.

In the latter case both elements showed very similar mass fractionation behavior, while introduction of a dry aerosol into the ICP by laser ablation or desolvation of the aerosol from the nebuliser is accompanied by greater dependence on operating conditions and matrix composition.

[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 **47**

Probing conformations of peptide ions in the gas phase by ion mobility and tandem mass spectrometry

Hisham Ben Hamidane[1], Florian Albrieux[2,3], Fabien Chirot[3], Florent Calvo[2], Rodolphe Antoine[2], Philippe Dugourd[2], Jérôme Lemoine[3], Yury O. Tsybin[1]

[1]LSMB, Ecole Polytechnique Fédérale de Lausanne, Switzerland
[2]LASIM-CNRS, Université Lyon 1, France.
[3]LSA-CNRS-Université Lyon 1, France.

Biological activity of peptides and proteins depends on their conformation. Despite a recent progress in analytical techniques development for peptide and protein conformation analysis, further improvement is required. Electron capture dissociation (ECD) is a recent tandem mass-spectrometry (MS/MS) technique used for peptide sequencing and post translational modification analysis. Here we report on the potential of ECD for structural characterization beyond primary structure. Fragmentation patterns are correlated to gas phase conformations suggested by ion mobility mass spectrometry (IM-MS) and replica exchange molecular dynamics (REMD). For that purpose, structurally relevant bioactive peptides have been used, ranging from 11 amino acids long substance P variants containing α, α-disubstituted amino acids to a 27 amino acid long stabilized alpha helix from the BH3 domain of the BID protein. Particular interest was devoted to a 25 amino acids long transmembrane domain of the influenza virus A M2 protein in its wild type form as well as in 6 structurally relevant variants. The ECD MS/MS experiments were performed on a 12 T ESI LTQ FT-ICR MS and IM-MS measurements were performed on a custom built ESI-IM-MS-qTOF MS. Preliminary data shows that similar ECD fragmentation patterns belong to the same conformational groups determined by IM-MS, suggesting a probable correlation between gas phase structure and radical mediated dissociation products. Furthermore structural constraints present in non natural amino acids containing a disubstituted C_α have been probed by ECD. Finally intramolecular cross linking obtained by metathesis of a pair of disubstituted olefinic residues demonstrates a product ion yield modulation that enables to distinguish the initial from the final state of that isobaric reaction.

Analytical Chemistry **48**

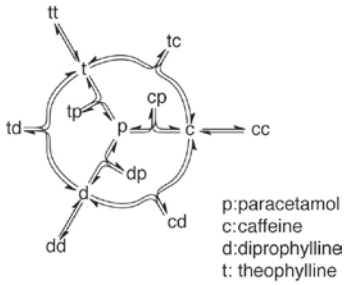
Evalution of 10 ppm HSQC NMR spectra to study complex systems at the fast exchange regime.

Application to the study of the association constants of xanthines

Rupali Shivapurkar[1], Youssef Achache[1], Eric Doelker[2], Damien Jeannerat[1]

[1] Department of Organic Chemistry, University of Geneva, 30, Quai Ernest-Ansermet, CH-1211, Switzerland
[2] School of Pharmaceutical Sciences, University of Geneva, 30, Quai Ernest-Ansermet, CH-1211, Switzerland

When molecules are weakly associated through hydrogen bounding, π-stacking, etc. the exchange is fast on the NMR time scale. The observed chemical shifts are therefore the weighed averages of the chemical shifts of the different forms in presence. When only two forms exist, for example when a molecule forms a dimer or when two molecules associate, a simple concentration dependence of the chemical shifts provide the sole constant. We wish to explore the more complicated situations of a small library of interacting drug molecules. We studied the interaction of xanthines (caffeine, theophylline and diprophylline) and how this mixture is influenced by the addition of paracetamol.



System studied using 10 ppm HSQC spectra

Analytical Chemistry **49**

Exploring the Impact of the Time-Temperature Roasting Profile on the Flavor of Coffee

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

The time-temperature roasting profile is thought to have a major influence on the flavor of the roasted coffee [1-3]. On-line analysis by Proton-Transfer-Reaction Time-of-Flight Mass-Spectrometry (PTR-ToF-MS) of five roasting profiles, ranging from high temperature short-time to low temperature long-time roasting, gave insight in real-time dynamic release of volatile flavor compounds (VOCs). Off-line analyses of the coffee brew via Headspace Solid Phase Micro Extraction Gas Chromatography Mass Spectrometry (HS SPME GC/MS) and sensory evaluation complemented these measurements. The PTR-ToF-MS measurements revealed differences in the release dynamics of VOCs and a strong modulation of the release dynamics by the time-temperature roasting profile. The off-line GC and sensory data of the coffee brew showed only minor differences. It is believed that the dark roast degree led to a leveling of the differences among roast profiles.

[1] Yeretzian, C.; Jordan, A.; Brevard, H.; Lindinger, W. *In ACS Symposium Series 763*; *Roberts, D. D., Taylor, A. J., Eds.; ACS: Washington, DC,* **2000**; 112-123.
[2] Moon, J.K.; Shibamoto, J.K.; *J. Agric. Food Chem.*, **2009**, *57(13)*, 5823-5831.
[3] Baggenstoss, J.; Poisson, L.; Kaegi, R.; Perren, R.; Escher, F.; *J. Agric. Food Chem.,* **2008**, *56(14)*, 5836-5846.

Analytical Chemistry **50**

Nose-Space Analysis of Coffee by on-line Proton-Transfer-Reaction Time-of-Flight Mass Spectrometry (PTR-ToF-MS)

Andrea F. Wild[1], Alexia N. Gloess[1], Lucio D'Ambrosio[1], Hans Rentsch[2], Adrian Rink[2], Chahan Yeretzian[1]

[1] Zurich University of Applied Science (ZHAW), Institute of Chemistry and Biological Chemistry, 8820 Wädenswil, Switzerland
[2] Delica AG, Hafenstrasse 120, CH-4127 Birsfelden

In recent years, headspace (HS) analysis has become one of the methods of choice for measuring volatile flavor compounds (VOC) in foods. There are obvious reasons for this. While essentially all food products emit VOC, these volatiles in the HS can be related to important properties of foods like flavor, age, geographic origin or history of treatment. One additional reason of relevance to this work is that the VOC in the HS represent those with sufficient volatility in the food to be present in the mouth's air during eating. VOC released from food during eating enter the breathing air-stream. Upon exhaling, these compounds move from the mouth to the nasal cavity retronasally, where they stimulate the sense of smell. While HS analysis of food gives reproducible data that enables many types of studies, direct analysis of air exhaled from humans during eating, i.e. Nose-Space (NS) analyses, is more representative of the changes in the food matrix that occur upon mastication, salivation, and temperature changes. Indeed, the NS profile reflects much closer the aroma that is perceived by consumers than the HS. Further, it permits examining the temporal evolution of the flavor [1].

Following the pioneering work of Taylor & co-workers, using API-MS (**A**tmospheric **P**ressure **I**onization), Yeretzian & co-workers have initially improved the technology by coupling the NS device to PTR-MS (**P**roton-**T**ransfer-**R**eaction) [1]. Here we report on a newly introduced setup for NS analysis with significantly improved mass resolution and sensitivity, using PTR-ToF-MS (time-of-Flight MS). Results on coffee are discussed.

[1] Yeretzian, C.; Pollien, P.; Lindinger, C.; Ali, A.; *Comprehensive Reviews in Food Science and Food Safety (CRFSFS),* **2004**, *3(24),* 217

Analytical Chemistry **51**

Measuring Partition Coefficients of VOCs and their Temperature Dependence by Dynamic Stripping and Proton-Transfer Reaction Time-of-Flight Mass Spectrometry

Flurin Wieland, Alexia N. Gloess, Chahan Yeretzian

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

Air-water partition coefficients play a significant role in understanding daily processes like aroma release from food products in various environmental phenomena or medical analysis (breath analysis). The equilibrium of gas dissolved in a liquid (e.g. water) can be described via Henry's Law constant (HLC).

We have developed and validated a dynamic approach using a stripping cell configuration coupled on-line to a Proton-Transfer-Reaction Time-of-Flight Mass Spectrometer (PTR-ToF-MS),[1-2] to measure with high accuracy the HLC of VOCs. This methodology allows the rapid determination of water-air partition coefficients, even for molecules with low volatility, and over an extended temperature range (25-90 °C).

We first discuss and validate several critical analytical aspects of the approach, in order to achieve highest accuracy and precision. We then present a series of detailed studies on the temperature dependence of the HLC for selected (coffee) aroma compounds.

[1] Pollien, P.; Jordan A.; Lindinger, W.; Yeretzian C.; *Int. J. Mass Spectrom.* **2003**, *228*, 69.
[2] Karl, T.; Yeretzian, C.; Jordan, A.; Lindinger W.; *Int. J. Mass Spectrom.* **2003,** *223/224*, 383.

Analytical Chemistry **52**

Effect of Coffee Extraction Method on Quality of Coffee

Alexia N. Gloess[1], Lucio D`Ambrosio[1], Barbara Zurfluh[1], Babette Kloppro gge[1], Karin Gerber[2], Annette Bongartz[2], André Strittmatter[3], Markus Rast[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]Rast Kaffee AG, 6031 Ebikon, Switzerland

Coffee is a very popular beverage all over the world. The way of preparing a coffee brew, however, varies from country to country and from individual to individual [1]. In this study, we compared nine wide-spread extraction methods of coffee brew, four espressi and five lungi, namely (i) espresso and (ii) lungo extracted with a semiautomatic espresso machine (Dalla Corte), (iii) espresso and (iv) lungo extracted with a fully automatic coffee machine (Schaerer), (v) Nespresso Arpeggio, (vi) mocha percolator (Bialetti), (vii) french press (Bodum), (viii) Bayreuth coffee machine (traditional Karlsbad method) and (ix) filter coffee. Analytical measurements of headspace aroma, acidity, titrable acidity, fat content, total solids, °Brix and content of caffeine and chlorogenic acids of the respective extracts are combined with sensory attributes. This allows us to correlate the individual aspects, describing a good cup of coffee, and to elucidate the pro's and con's of the respective brewing techniques.

[1] Petracco, M. *In Coffee: Recent Developments, Clarke, R. J., Vitzthum, O. G., Eds.; Blackwell Science: London*, **2001**; 140-164.

Analytical Chemistry **53**

How much Robusta Coffee is in a Roasted Coffee Blend? Quantification of the Robusta Fraction via two Alternative Instrumental Methods: Confocal Raman Spectroscopy and HPLC

Lucio D'Ambrosio, Thomas Wermelinger, Babette Klopprogge, Chahan Yeretzian

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

More than 80 species of the genus *Coffea L.* (Rubiaceae) are know, of which two are of commercial significance: *Coffea arabica*, colloquially called "Arabica", accounts for 75% of world production, and *Coffea canephora*, called "Robusta", accounts for 24 %. Since Arabica is generally considered of higher quality and demands higher prices that Robusta, fraudsters have been tempted to falsify the product declaration.

International coffee trade is conducted almost exclusively with green coffee, in which case Arabica and Robusta can easily been distinguished (e.g. by their exterior appearance such as size or shape, or by their genetic make-up). Yet, once roasted, identification of Robusta in a blend and quantification of the blending ratio Arabica/Robusta is much more difficult. Sensory testing by highly experienced coffee tasters may alloy to get a rough estimate of the mixing ratio. Yet there is a need and commercial interest in having objective and precise analytical methods of quantification of the Robusta fraction in a blend.

The aim of this work is compare two alternative instrumental approaches that have been reported in the literature. Both are based on the analysis of the lipid composition. While the HPLC approach focuses on 16-O-methylcafestol, only found in Robusta beans [1], the confocal Raman spectroscopic approach takes advantages of differences in the spectroscopic signature of Robusta vs. Arabica (e.g. the Kahweol adsorption) [2].

[1] Pettitt, B.C.; *J. Agric. Food Chem.* **1987**, *35*, 549.
[2] Rubayiza, A.B.; Meurens, M.; *J. Agric. Food Chem.* **2005**, *53*, 4654.

Analytical Chemistry **54**

Rapid Estimation of Total Polyphenols in Coffee Brews by Flow Injection Analysis with Colorimetric Detection

Sergio Petrozzi, Lucio D'Ambrosio, Chahan Yeretzian

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

Polyphenols are of large abundance in coffee [1] and contribute to its flavour and health properties. The objective of this work was to develop a fast, low cost and robust method for the quantification of polyphenols in coffee brew. In contrast to alternative approaches, which require sample preparation and separation, a flow-injection analysis (FIA) system with colorimetric detection for the estimation of the total amount of polyphenols (expressed as gallic acid equivalent) is described. After the development and optimization of the FIA setup [2], the spectrophotometric characteristics of various equivalent chemicals, such gallic acid (commonly used as universal reference-standard polyphenol compound [3]), were evaluated with three alternative phenol determination methods. For the analytical procedure and FIA design suggested in this work, the Folin-Ciocalteu method [4] was found to be superior to the Folin-Denis method in terms of costs and to the 4-Aminoantipyrine method in terms of sensitivity and negligible need for colorimetric correction. The major non-phenolic coffee components gave no colour contribution in the Folin-Ciocalteu reaction. Under the optimized conditions the proposed system was applied to the estimation of total polyphenols in coffee brews prepared with ground coffee (species *C. arabica* and *C. robusta*) resulting in a sample throughput of approximately 110 samples per hour.

[1] Shahidi, F.; Naczk, M.; *Phenolics in Food and Nutraceuticals*, CRC Press, **2004**, ISBN: 978-1-58716-138-4.
[2] Karlberg, B.; Pacey, G. E.; *Flow Injection Analysis, A Practical Guide*, Elsevier; Amsterdam, **1989**.
[3] Proteggente, A. R. et al.: *Free Radical Research*, **2005**, *36*, 217-233.
[4] Folin, O.; Ciocalteu, V.; *J. Biol. Chem*., **1927**, *73*, 627-650.

Analytical Chemistry **55**

Rapid quantitative analysis of jasmonic acid in plant extracts by LC-MS/MS

Adeline Chauvin[1], Gaetan Glauser[1], Edward Farmer[2], Jean-Luc Wolfender[1]

[1]School of Pharmaceutical Sciences, University of Geneva, University of Lausanne, CH-1211 Geneva 4, Switzerland, [2] Plant Molecular Biology, University of Lausanne, Biophore, CH-1015 Lausanne, Switzerland.

Liquid chromatography coupled to mass spectrometry (LC-MS) is a powerful tool for metabolite quantification in complex matrices. However, matrix effects are a common issue with atmospheric pressure ionization sources and can affect the quantitative performance of the detector. Generally, isotopically labeled internal standards compensate quite well for these effects, although it is not always sufficient. In this study a new method using LC-MS/MS was devised for the rapid quantification of jasmonic acid (JA) in extracts of the plant *Arabidopsis thaliana*. JA is a key hormone that plays various roles in defense responses [1]. To shorten run times and minimize ion suppression, the LC separation was optimized based on fused core particle technology [2]. An isotopically labeled internal standard (^{18}OJA) presenting identical retention time to JA was used. Specific transitions were selected in negative ion mode for JA (*m/z* 209->59) and the internal standard (*m/z* 213->63). Calibration curves performed in pure solvents and in matrices consisting of extracts from *Arabidopsis* insertion mutants lacking JA were evaluated. The limit of quantification (LOQ) was determined to be 5 pmol·g^{-1} fresh weight. This method will enable the quantification of low levels of JA in unstressed and stressed plants, including new point mutants in which JA production is impaired.

Acknowledgment: Funding is provided by SNF Grant no. 205320-124667/1

[1] E. E. Farmer, *Nature* **2007**, *448*, 659.
[2] J. M. Cunliffe, T.D. Maloney*, J. Sep. Sci*. **2007**, *30,* 3104

Analytical Chemistry **56**

A molecular dynamics study of water molecules with silica surface in chromatographic system

Prashant Kumar Gupta, Marek Orzechowski and Markus Meuwly*

Department of Chemistry, University of Basel, Klingelbergstrasse 80, CH-4056 Basel

Besides its function as a solvent, water is known to play an active functional role in biological systems. However, other complex systems have been found to be also influenced by the amount and presence of water, including chromatographic systems. Similar to lipid hydration, there is a heterogenous distribution of water molecules between the two silica layers. The ordering and dynamics of water molecules differs due to different interfacial regions within the system [1],(-OH groups of silica surface and the hydration sites in lipid bilayers)[2]. Due to this reorganisation dynamics, the investigation of such a system by standard means (X-ray or NMR spectroscopy), becomes difficult[3].

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 and mobile phase is carried out by molecular dynamics (MD) simulations. In this work, we investigate the disordered structure and the exchange dynamics of water molecules and the influence of solvent composition on the stability and dynamics of water molecules [4]. Such an approach reveals the temporal evolution of this complex system at the atomistic level.

[1] Scott R., *Faraday Symp. Chem. Soc.*, **1980**, *15*, 49.
[2] Finer E., Darke A., *Chem. Phys. Lipids*, **1974**, *12*, 1.
[3] E. Von Goldammer , and H. G. Hertz, *J. Phys. Chem.*, **1970**, *74*, 3734.
[4] J. Braun, A. Fouqueau, R. J. Bemish and M. Meuwly, *PCCP*, **2008**, *10*, 4765-4777.

Analytical Chemistry **57**

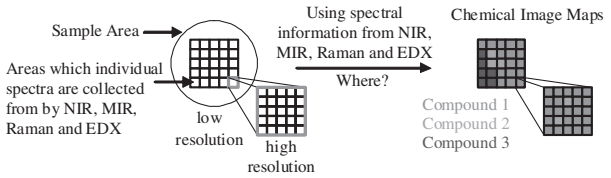
Chemical Imaging of Solid Dosage Forms. The Synergy of NIR, MIR, Raman, and EDX mapping

Christian Adlhart[1,2], Thomas F. Haefele[3], Kurt Paulus[3], and Yusuf Sulub[4]

[1]Ciba Expert Services, 4002 Basel, Switzerland
[2] Zurich University of Applied Science (ZHAW), 8820 Waedenswil, Switzerland (current address)
[3]Novartis Pharma AG, 4002 Basel, Switzerland
[4]Novartis Pharma AG, East Hanover, NJ 07936-1080, USA

Chemical imaging (CI) complements chemical information with spatial information. Even though CI is successfully applied in pharmaceutical industry, only few applications are reported combining different spectroscopic techniques to obtain a detailed picture of heterogeneous mixtures [1]. The combined approach enables identifying and visualizing more ingredients within a formulation than accessible with a single spectroscopic technique.

Identical samples of four commercially available tablets (ASS 100 Hexal®, Voltaren® Dispers, Dolodoc® 200 mg and Melabone® K) containing up to three active pharmaceutical ingredients (APIs) and up to ten different components were analyzed by NIR, MIR, Raman, and EDX mapping. The results of large area and high resolution images are discussed by means of collocation, detectability, chemical speciation, shape, morphology, bulk and find distribution as well as total concentration of the components.



[1] Clarke F.C., Jamieson M.K., Clark D.A., Hammond S.V., Jee R.D., Moffat A.C., *Anal. Chem.* **2001**, *73*, 2213.

Analytical Chemistry **58**

The Infrared Beamline at the Swiss Light Source: a Tool for Chemical Microanalysis

L. Quaroni[1], J. Schneider[1], D. Armstrong[1], J. Wambach[2], P. Lerch[1]

[1]Swiss Light Source, [2]Department of Energy; Paul Scherrer Institut, 5232 Villigen PSI, Switzerland

The Infrared beamline at the Swiss Light Source (beamline X01DC) has been conceived as a multidisciplinary facility for spectroscopy and spectromicroscopy using infrared synchrotron radiation. The beamline layout accommodates four different branches, each dedicated to a specific experimental setup.
An infrared microscopy branch is operated as a service to the national and international scientific community, and has been opened to external users since January 2009. The endstation consists of a Bruker Hyperion 3000 microscope coupled to a Vertex 70 interferometer. The microscope takes advantage of the brightness of synchrotron emission in the mid infrared region to overcome the throughput limitation of IR microscopes when used close to the diffraction limit. As such it is an effective tool for performing spectromicroscopy and mapping experiments on small samples, in the micrometer size range. Furthermore, high flux and brightness in the far infrared region are used for both spectroscopy and high resolution microscopy. [1] Applications cover a wide variety of disciplines, including material science, biochemistry and cell physiology, biomedical sciences and diagnostics, polymer chemistry, forensics, conservation, geochemistry and astrochemistry.

[1] Michael C. Martin, Ulrich Schade, Philippe Lerch, and Paul Dumas; *Trends Anal. Chem.* **2010**, in press.

Analytical Chemistry **59**

Chemotaxonomic study of medicinal *Viola* sp. from Switzerland based on high resolution UHPLC-TOF-MS metabolite profiling

Hakim Kratou, Philippe J. Eugster, Jean-Luc Wolfender*

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

The herbaceous and hardy plants of the *Viola* genus including violets and pansies belong to the Violaceae family. In Switzerland, 27 species and 4 subspecies are growing wild. Among them, *Viola tricolor* L. (wild pansy) and the taxonomically closely-related plant *Viola arvensis* Murray (field pansy) have a medicinal interest. In the local folk and traditional medicine, their flowering aerial part has been widely used for their cleansing, depurative, anti-inflammatory, analgesic and antipyretic properties. These properties are ascribed to the presence of the plant secondary metabolites such as flavonoids, mucilage, phenolic acids derivatives, carotenoids and coumarins [1-4]. The description of most of these constituents is however rather vague. Therefore, the aim of this study was to develop a high resolution profiling method based on ultra high pressure liquid chromatography coupled to time-of-flight mass spectrometry (UPLC-TOF-MS) for a comprehensive survey of the secondary metabolite composition [5]. The dereplication was performed based on the extracted molecular formula and UV spectra obtained on-line, cross search with chemotaxonomic information from the literature. This profiling method was used to compare different medicinal *Viola* species to other wild species collected in Switzerland. This approach enabled a precise chemotaxonomic comparison of closely related taxa from the *Viola* species and revealed their very complex composition.

[1] V. Vukics, T. Ringer, A. Kery, *J. Chrom. A* **2008**, 1206(1), 11-20.
[2] A. Toiu, L. Vlase, I. Oniga, *Chem. Nat. Compd.* **2008**, 44(3), 357-358.
[3] E.I. Zabaznaya, *Khim. Prir. Soedin* **1985**, 1, 116.
[4] K.D. Mishnyeva, T.M. Hontova, O.P. Khvorost, *Med. Khim.* **2006**, 8(4), 35-37.
[5] J.L. Wolfender, *Planta Med.* **2009**, *75*, 719.

Analytical Chemistry **60**

Rapid identification of mycoalexins from human pathogenic fungi using HPLC-UV-MS offline coupled to microflow NMR at the Petri dish level

Nadine Bohni[1], Katia Gindro[2], Philippe J. Eugster[1], Olivier Schumpp[2], Michel Monod[3], Jean-Luc Wolfender[1]

[1]School of Pharmaceutical Sciences, University of Geneva, University of Lausanne, Quai Ernest-Ansermet 30, 1211 Geneva, Switzerland
[2]Mycology group, Agroscope Changins ACW, Route de Duillier, 1260 Nyon, Switzerland
[3]Service for Dermatology and Venereology, Laboratory of Mycology, CHUV, 1011 Lausanne, Switzerland

Onychomycosis is the most prevalent nail disease. Although dermatophytes are the main cause of onychomycosis, *Fusarium* spp. and various other non-dermatophyte filamentous fungi are often isolated from abnormal nails. The aim of this study is to investigate the interactions of different fungal species from nails or plants and search for stress induced metabolites (mycoalexins) [1] that might be lead compounds for new antifungal agents. The screening of mycoalexins is performed on fungi in confrontation directly at the Petri dish level using sensitive UHPLC-TOFMS. To find new molecules or to assign constitutional isomers, isolation on a larger HPLC scale is essential. By direct coupling of semi-preparative HPLC to UV and MS, the fractionation process is directly monitored. Through offline coupling with microflow NMR (CapNMR™) equipped with automated sample injection (One Minute-NMR™) [2] the structural analysis of the metabolites down to the microgram level is possible. The natural product discovery platform is presented. The methods were applied to the crude extract of co-cultured human pathogenic fungi isolated from patients with onychomycosis at CHUV, to target novel and/or bioactive natural products.

Acknowledgment: SNF Sinergia project CRSII3_127187/1

[1] G. Glauser, K. Gindro, J. Fringeli, J.-P. de Joffrey, S. Rudaz, J.-L. Wolfender, *J. Agric. Food Chem.* **2009**, *57*, 1127.
[2] J.F. Hu, G.R. Eldridge, Y.H. Yu, M. O'Neil-Johnson, *Progr. Chem.* **2008**, *20*, 429.

Analytical Chemistry **61**

Fingerprinting method of an extract of *Morinda citrifolia* (Rubiaceae) from French Polynesia by Ultra High Pressure Liquid Chromatography Time-Of-Flight Mass Spectrometry

Raimana Ho[1], Olivier Ciclet[1], Philippe J. Eugster[1], Phila Raharivelomanana[2] and Muriel Cuendet[1]

[1]School of Pharmaceutical Sciences, University of Geneva, Quai Ernest-Ansermet 30, CH-1211 Geneva 4, Switzerland
[2]Biodiversité Terrestre et Marine, University of French Polynesia, BP 6570, 98702 Faa'a, Tahiti, French Polynesia

In French Polynesia, *Morinda citrifolia* L. (Rubiaceae) known as *Nono*, is a small shrub belonging to the Rubiaceae family. In the past, the Polynesians used the roots of *M. citrifolia* as a dye [1]. Moreover, there is a long history of the use of *M. citrifolia* as an important medicinal plant for the treatment of asthma, bone fractures, cancer, urinary difficulties and many other ailments [2-3]. Recent studies have shown that various parts of *Nono* contain benzophenones and anthraquinones, these last exhibiting an induction of quinone reductase [4]. The objective was to develop a fast and reproducible analytical method for the observation of characteristic fingerprint of the anthraquinones. The $CHCl_3$-soluble extract of the bark of *M. citrifolia* was analyzed by an ultra high pressure liquid chromatography coupled to time-of-flight mass spectrometry (UHPLC-TOF-MS) method to detect the fractions rich in anthraquinones comparing by elemental composition. The isolation of the interesting compounds was carried out by different chromatographic methods and finally the pure compounds were tested for their cancer chemopreventive activity.

[1] P Pétard, *Plantes Utiles de la Polynésie-Raau Tahiti*, **1986**, Editions Haere Po No Tahiti, Papeete.
[2] O Potterat et al., *Planta Med*., **2007**, *73*, 191.
[3] AD Pawlus et al., *J. Pharm. Pharmacol*., **2007**, *59*, 1587.
[4] Y Deng et al., *J. Nat. Prod*., **2007**, *70*, 2049.

Analytical Chemistry **62**

Deamidation and transamidation of substance P revealed by Fourier transform ion cyclotron resonance mass spectrometry

Luca Fornelli[1], Adrien W. Schmid[1], Luigino Grasso[2], Horst Vogel[2] and Yury O. Tsybin[1]

[1]LSMB, Ecole Polytechnique Fédérale de Lausanne, Switzerland
[2]LCPPM, Ecole Polytechnique Fédérale de Lausanne, Switzerland

Recent *in vitro* studies indicate that the formation of aggregates of specific peptides and proteins observed in different neurodegenerative disorders could be driven by post-translational modifications. Particularly, enzymatically-induced intra- and intermolecular cross-linking and deamidation might play an important role in the oligomerization of amyloid-beta and alpha-synuclein. In the present study we used neuropeptide Substance P (SP, $RPKPQQFFGLM\text{-}NH_2$) as a model to elucidate the catalytic activity of the ubiquitously expressed transferase tissue transglutaminase (tTGase). Under specific reaction conditions, tTGase can induce *in vitro* both peptide deamidation and cross-linking, as revealed by reverse-phase liquid chromatography high-resolution Fourier transform ion cyclotron resonance mass spectrometry. *Electron capture dissociation* (ECD) is a tandem mass spectrometry technique which produces peptide fragmentation at the backbone level mainly, retaining therefore post-translational modifications and amino acid lateral chains. We used ECD to clarify the sequential deamidation mechanism of the two glutamines of SP, with residue Glu_5 being deamidated first and Glu_6 only after it, and also determine the precise structure of different populations of SP cross-linked dimers. The developed analytical methodology, accompanied by *in vivo* cellular assay data, will be also employed for other biologically relevant neuropeptides, including truncated amyloid-beta peptides.

Analytical Chemistry **63**

Initial development of an aptamer-aided MALDI detection method for adenosine

Stephan R. Fagerer[1], Pawel L. Urban[1], Andrea Amantonico[1], Yu-Chie Chen[2], Renato Zenobi[1]

[1] Department of Chemistry and Applied Biosciences, ETH Zurich CH-8093 Zurich, Switzerland
[2] Department of Applied Chemistry, National Chiao Tung University, Hsinchu, Taiwan

Metabolomic studies of single cells rather than bulk studies have several advantages. For instance, an inhomogeneous population may give rise to misleading averaged data. While the need for reliable, quantitative single-cell methods is widely acknowledged, few are reported in the literature. This is mainly due to the minute amount of metabolites that can be obtained from a single cell. Here, the initial development of an ultrasensitive adenosine assay with signal amplification by aptamer-binding and mass spectrometric readout is presented.

It utilizes an aptamer system possessing a high affinity for adenosine. In the presence of the target molecule two different DNA oligonucleotides form a pocket between each other to bind two adenosine molecules. The first aptamer is immobilized on a glass slide while the second aptamer is immobilized on a gold nanoparticle. The final readout is the laser desorption/ionization signal from single gold ions (Au^+).

Due to the fact that a single binding event may result in the detection of a high number of reporter ions we anticipate a high sensitivity of the final method. In order to estimate the amplification factor the surface density (obtained by SEM) and the corresponding LDI spectra were correlated and it has been found that potentially a few hundred molecules of adenosine could be detected.

Analytical Chemistry **64**

Accelerated Decay of Peroxynitrite in Presence of 1,2-Aminoalcohols

Christian Molina, Reinhard Kissner, Willem H. Koppenol

ETH Zürich, Laboratorium für Anorganische Chemie, Wolfgang-Pauli-Str. 10, CH-8093 Zürich

Peroxynitrite and its conjugated acid peroxynitrous acid are discussed to be formed and to cause oxidation in living tissue. [1]

In biochemical research, tris(hydroxymethyl)aminomethane, commonly known as Tris, is used widespread as buffer substance and assumed to be almost inert.

Kinetic experiments showed that increasing concentrations of Tris lead to increased decay rates of peroxynitrite in aqueous solutions at neutral pH and room temperature.

While neither alcohols nor amines show this effect in general, 2-aminoethanol, a 1,2-aminoalcohol like Tris, does accelerate the decay of peroxynitrite too.

This and the results of further experiments with Tris analogues will be presented and discussed

[1] W. H. Koppenol, J. J. Moreno, William A. Pryor, H. Ischiropoulos, J. S. Beckman, *Chem. Res. Toxicol.* **1992**, *5*, 834

Analytical Chemistry **65**

Human Caspase Specific DARPin Characterization using Microarray and SPR Analysis

Jens Sobek[1] *, Thilo Schröder[2], Stefan Schauer[1], Ralph Schlapbach[1], Markus Grütter[2]

[1] Functional Genomics Center Zürich, Winterthurerstr. 190, CH-8057 Zürich, Switzerland
[2] University of Zürich, Winterthurerstr. 190, CH-8057 Zürich, Switzerland

Caspases play a fundamental role in apoptosis and inflammation, however there are no techniques available to specifically monitor caspase activity in apoptotic or inflammatory cells. Studies based on commercially available short peptide-based substrates lack on proposed specificity [1]. We have developed a protein microarray using Designed Ankyrin Repeat Proteins (DARPins) [2] as capture reagents to specifically detect and monitor caspase activity in cells. Due to their favourable biophysical properties DARPins represent ideal binding proteins for protein array applications. We have established expression and purification protocols for caspase-1 to caspase-9 designed for selection of DARPins using ribosome display [3,4]. DARPins have been selected against caspase-1 to caspase-8 and are currently undergoing further evaluation. Array experiments demonstrate that DARPins are functional and maintain their binding capability once they are immobilised at surfaces.

In our study we have developed a new generation of improved protein microarrays based on DARPins as capture reagents. Surface plasmon resonance (SPR) experiments were performed to determine binding constants of selected DARPins against caspase-7 and are compared to results obtained with microarrays.

[1] G.P. McStay, G.S. Salvesen, D.R. Green Cell Death Differ. 2008, 15, 322.
[2] H.K. Binz, M.T. Stumpp, P. Forrer, P. Amstutz, A. Plückthun J. Mol. Biol. 2003, 332, 489.
[3] H. Roschitzki-Voser, T. Schroeder, submitted.
[4] C. Zahnd, P. Amstutz, A. Plückthun Nature Methods 2007, 4, 269.

Analytical Chemistry **66**

Accelerating Analytical Sample Preparation - Faster and Better by Flexible and Modular Automated

Amira Abou-Hamdan

Chemspeed Technologies, Rheinstrasse 32, 4302 Augst, Switzerland

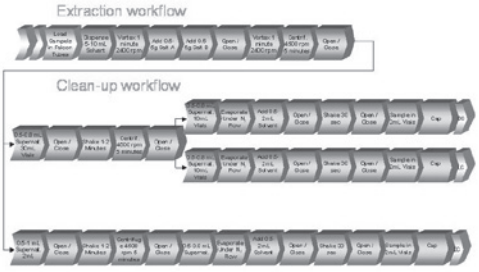
The general need for increased efficiency in the laboratory has kick-started the development of innovative equipment for workflow automation.

Many laboratories within the chemical industry in the meantime have adopted the use of automated Technologies, which has been boosting productivity in the pharmaceutical industry since several decades.

For this reason instruments similar to the equipment, which originally has been designed to accelerate the process of Drug Discovery, now commonly are being used for parallel, unattended Sample Preparation, Pretreatment, Digestion and Analysis. Multiple dispensing of solid and liquid samples, sample treatment with corrosive chemicals under harsh conditions, work-up steps like filtration, centrifugation & SPE purification are only some of the most relevant workflow steps.

Other technical requirements to these instruments might strongly differ from workflow to workflow. Generally a diverse set of complex unit operations needs to be automated, which are characteristic to each individual workflow. Only flexible, highly modular, and scalable equipment has the premise of covering the complex automation needs for faster and better analytical sample preparation.

Using a selection of case studies, this presentation shows how a variety of challenging parallel sample preparation workflows have been fully automated.Fully automated QuEChERS workflow for pesticide analysis Fully automated fatty acid analysis (accredited by UKAS)



Throughput = 96 food samples / 8h (4 x 24 samples campaigns)