doi:10.2533/chimia.2006.375
CHIMIA **2006**, *60*, No. 7/8

Analytical Chemistry **5**

Airborne Cell Chemistry & Nanoparticle based CEC

Staffan Nilsson

Applied Biochemistry, Lund Institute of Technology, Lund University, P.O.box, 124, S-221 00 Lund, Sweden.

New insights in biomedicine and related areas require the parallel development of new analytical methods. We have successfully developed a new technique for chemical analysis based on the use of levitated drops, suitable for the study of intra- and extracellular reactions at the single cell or few cell level. Microenvironments suited for specific cell types and cell reactions can be created in levitated drops to serve as biomimetic systems. The technique is now being adapted for use in combination with other miniaturised analytical methods like capillary electrophoresis (CE), capillary electrochromatography (CEC) and molecularly imprinted polymers (MIPs).

Cell-containing 100-500 nL drops are levitated in a specially designed ultrasonic field. Cells and reagents are added to the drop using flow-through dispensers, and the cell reactions are monitored using fluorescence imaging detection. The cells are subsequently lysed and extractions performed in the levitated drops. The drop or part of its contents is transferred into capillaries, after which the contents are separated by CE/CEC and detected using nano-ESI-MS. We have shown that the levitated method can be used to follow the lipolysis in primary adipocytes and cell-cell communication between adipocytes and B-cells.. We have also shown that micro extractions can be performed in the levitated drops. The drop or part of its contents is easily transferred into capillaries, after which the contents are separated by CE/CEC and detected using nano-spray ESI-MS. To detect molecules at the single cell level, we are now developing a mass spectrometer interface with extremely low detection limit.

Nanoparticle based CEC will be discussed as well. An alternative way to perform CEC, compared to traditional formats, is to use a pseudostationary phase (PSP). Dextran-coated nanoparticles have been used as PSP for highly efficient CEC separations (plate numbers up to 700 000 /m) of neutral analytes. The dextran coated nanoparticles role to suppress non-coated capillary wall adsorption of proteins will be shown and discussed.

Analytical Chemistry **6**

The use of ostracod shells as a paleothermometer by measuring Calcium isotope ratios and trace elements with ICPMS

Nadine Bohni[1], Volker Wennrich[2], Bodo Hattendorf[1], Detlef Günther[1]*

[1]ETH Zürich, Laboratory of Inorganic Chemistry, D-CHAB, CH-8093 Zürich, Switzerland
[2]University of Leipzig, Institute for Geophysics and Geology, D-04103 Leipzig, Deutschland

The shell chemistry of ostracods is often used in paleoenvironmental reconstruction. As elemental composition and isotope ratios depend on different indicators as water salinity or environmental temperature, a chemical analysis of these crustaceans can shed light on environmental changes [1].

In this work variations in the Calcium isotopic composition within ostracod samples covering a time span of 1000 years were studied. In order to allow a rapid screening, ICPMS with dynamic reaction cell was chosen. This technology allows measuring the most abundant isotope ^{40}Ca by neutralization of the interferences from Argon ions in a reaction with ammonia [2,3].

In addition LA-ICPMS was used to analyze the major, minor and trace elements in the ostracods, providing spatial and bulk information and especially to correlate temperature variations based on known thermometric indicators like the Mg/Ca concentration ratio [1].

[1] Holmes J., Chivas A., in: *The Ostracoda,* Holmes J., Chivas A. Eds., American Geophysical Union, **2002**, 185.
[2] Tanner S.D., Baranov V.I., Völlkopf U., *J. Anal. At. Spectrom.* **2000**, *15*, 1261.
[3] Hattendorf B., Wanner H., Gu H., Dorn S., Günther D., in *Plasma Source Mass Spectrometry: Current Trends and Future Developments*, Holland G., Bandura D. Eds., RSC Publishing, **2005**, 91.

Analytical Chemistry **7**

Microfabricated electrospray emitters for peptide and protein tagging

L. Dayon[a], M. Prudent[a], M. Abonnenc[a], J. Josserand[a], C. Roussel[a], N. Lion[a], A. Brajter-Toth[b], H.H. Girault[a*]

[a] Laboratoire d'Electrochimie Physique et Analytique, Ecole Polytechnique Fédérale de Lausanne, CH-1015 Lausanne, Switzerland
[b] University of Florida, Department of Chemistry, Gainesville, FL 32611, USA

Microspray emitters for mass spectrometry obtained by photoablation of polymer substrates are composed of microchannels with on chip microband electrodes [1].

Such microspray emitters have been used for electrochemical tagging to count cysteines in peptides [2] and probe cysteine reactivity in proteins [3]. During electrospray ionization mass spectrometry, tags are produced at the microband electrode by the oxidation of methoxycarbonyl-1,4-hydroquinone to the corresponding benzoquinone, which reacts with cysteines in the flow channel on chip. The tagged products are analyzed continuously by ion trap mass spectrometry. A new microspray chip with an on chip microband electrode array has been fabricated. Optimization of the performance of the chip with the microelectrode array for the tagging of cysteines in peptides will be described.

Multichannel microspray ionization chip emitters have also been designed for the development of analytical strategies based on tagging. The counting of specific amino acid in peptides, the relative quantification of peptide samples and the study of metal-peptide complexation will be presented as promising application of tagging in the multichannel device.

[1] T.C. Rohner, J.S. Rossier, H.H. Girault, *Anal. Chem.* **2001**, 73, 5353.
[2] L. Dayon, C. Roussel, M. Prudent, N. Lion, H.H. Girault, *Electrophoresis* **2005**, 26, 238.
[3] L. Dayon, C. Roussel, H.H. Girault, *J. Proteome Res.* **2006**, 5, 793.

Analytical Chemistry **8**

Determination of NH_3 in Ambient Air using Photoacoustic Spectroscopy

Ch. Hueglin, A. Fischer, P. Graf, B. Schwarzenbach, B. Buchmann

Laboratory for Air Pollution and Environmental Technology, Empa, Ueberlandstr. 129, 8600 Duebendorf, Switzerland

Ammonia is a relevant atmospheric pollutant. Deposition of gaseous ammonia and ammonium aerosol through dry and wet deposition contributes to both acidification and eutrophication of ecosystems. Ammonia has a relative short atmospheric lifetime and is deposited fairly close to its sources. It is able to neutralize acids and forming particulate ammonium, which can be transported over long distances. In order to assess the effects of reduction measures, as well as to study the role of ammonia on secondary aerosol formation processes, accurate and reliable measurement systems for ambient ammonia are needed.

A photoacoustic system designed for long-term measurement of ammonia in ambient air was developed in close cooperation with Omnisens SA (Lausanne, Switzerland). The system uses a CO_2 laser and a resonant photoacoustic cell for NH_3 detection at sub-ppb level [1]. To account for the signal of interfering species (i.e. H_2O and NH_3 resulting from thermal dissociation of ammonium nitrate) a set-up that consists of two sampling lines (reference and sample line) is used. The two sampling lines are identical, except that the reference line includes a citric acid coated diffusion denuder for selective removal of NH_3. A two-channel sampling system is used to switch between the two sampling lines. The ambient NH_3 concentration is derived from the difference of consecutive measurements of NH_3 in the reference and sample line. We present the set-up and applications of these instruments within the Swiss National Air Pollution Monitoring Network (NABEL).

[1] S. Schilt, L. Thévenaz., M. Niklès, L. Emmenegger, Ch. Hueglin. (2004). *Ammonia monitoring at trace level using photoacoustic spectroscopy in industrial and environmental applications*. Spectrochimica Acta Part A, 3259-3268.

Analytical Chemistry **9**

The micro-XAS Beamline at the Swiss Light Source (SLS): A New Analytical Facility Dedicated to Micro-beam Applications

A. M. Scheidegger, D. Grolimund, M. Harfouche, M. Willimann

Paul Scherrer Institute PSI, CH-5232 Villigen, Switzerland

Synchrotron-based X-ray absorption spectroscopy (XAS), X-ray fluorescence (XRF) and X-ray diffraction (XRD) have become key analytical techniques and have had a major impact on exploring the chemical reactivity and the structural identity of materials. In view of the importance to precisely characterize the physical and chemical attributes in complex heterogeneous materials on the micron scale, there has been a considerable effort to develop high-resolution analytical X-ray probes in the hard X-ray regime using state-of-the-art X-ray focusing devices. In this paper will present the layout of a new X-ray microprobe facility at the Swiss Light Source (SLS). The optical concept is optimized regarding micro-focusing with sub-micron ($<1\text{x}1\mu\text{m}^2$) spatial resolution using high resolution monochromatic beam of energies ranging from 4keV to ~18keV. Furthermore, the potential of micro-scale chemical speciation will be discussed and studies exploring molecular-level processes in complex and heterogeneous materials will be shown.

Analytical Chemistry **10**

A Two-Steps Strategy for the Efficient Detection and Localisation of Stress-Induced Metabolites in *Arabidopsis Thaliana* by UPLC-TOF-MS

E. Grata[a,b], D. Guillarme[b], J. Boccard[b,c], G. Glauser[a,b], P.A. Carrupt[c],
J.-L. Wolfender[a] and S. Rudaz[b]

LPP[a], LCAP[b], LCT – Pharmacochimie[c], School of Pharmaceutical Sciences,
University of Geneva, University of Lausanne, 30 quai Ernest-Ansermet,
CH-1211 Geneva 4, Switzerland.

An LC-MS metabolomic strategy has been developed in order to obtain the most comprehensive possible survey of the metabolome modifications that occur in *A. thaliana* upon leaf wounding [1]. The LC-MS analyses were performed according to a two-steps strategy: rapid screening and precise localisation. In the first step (screening), a rapid UPLC-TOF-MS gradient on a short column (50x1.0 mm i.d.) was used to highlight significant variations between numerous plant specimens (control versus wounded). The LC-MS data were treated as Total Mass Spectra (TMS) where the LC dimension was omitted. PCA performed on the TMS demonstrated a clear discrimination between the different groups and provided a list of the most discriminant ions (m/z). In the second step (localisation), the ions detected in the screening procedure were precisely identified with high peak capacity UPLC columns. The screening gradient was precisely transferred on very long columns (two 150 x 2.1 mm i.d.) and allowed a baseline separation of more than 200 peaks while only 30 were observed with the rapid procedure. The separation of various isomers was thus obtained and thanks to the high mass accuracy of TOF-MS, isobaric compounds were differentiated. This two-steps strategy has permitted to detect new wound-inducible low molecular mass regulators which might be involved in plant defence signalling.

Acknowledgements: SNSF is thanked (grant 205320-107735 to JLW).

[1] A. Thiocone, E. Grata, J. Boccard, P.A. Carrupt, S. Rudaz, J.-L. Wolfender, *Chimia*, **2005**, *59*, 362.

Analytical Chemistry **11**

Nanometer Scale Topographic and Chemical Imaging of Biofilms by Atomic Force Microscopy and Raman Spectroscopy

Thomas Schmid, Johannes Burkhard, Renato Zenobi

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

Biofilms are aggregates of microorganisms and extracellular polymer substances (EPS) that occur at interfaces of aqueous systems. EPS are microbially generated biopolymers, such as polysaccharides and proteins, which form a hydrogel matrix that allows the formation of stable microcolonies. Biofilms are the most common life form of bacteria in nature and play an important role in various technical processes (e.g. wastewater treatment plants, biofouling in industrial plants). For optimization of biocides and wastewater treatment, a detailed knowledge of structure and composition of the hydrogel is necessary. In this study, a combination of atomic force microscopy (AFM) for nanometer scale imaging and Raman spectroscopy for chemical characterization is employed. In tip-enhanced Raman spectroscopy (TERS), noble metal-coated AFM tips are introduced into the laser focus of a Raman microscope in order to enhance the Raman signal and to improve the lateral resolution down to a few tens on nanometers.

AFM measurements revealed the potential of the setup for high-resolution imaging of soft biological matter. The hydrogel-forming polysaccharide alginic acid and its salts were used as model substances for bacterial EPS. Here, AFM images revealed a network-like structure of alginate with water-filled pores with diameters ranging from < 100 nm up to a few 100 nm. In biofilms grown in river water, EPS matrix and bacteria could be visualized, where even small features such as flagella and pili were clearly visible. Raman signals of alginate could be enhanced by Ag or Au colloids inside or on top of alginate hydrogels and by Ag-coated AFM tips in TERS measurements. The aim of further experiments is to image and discriminate different biopolymers (e.g. polysaccharides and proteins) in hydrogels, biofilms, and other biological matrices with nanometer scale spatial resolution.

Analytical Chemistry **12**

Do we need a master in Analytical Science? An example in Lyon

Jérôme Randon

Laboratoire des Sciences Analytiques, Université Claude Bernard Lyon,
43 Bd du 11 Novembre 1918, 69622 Villeurbanne cédex, France

Increasing the number of students in the higher education system, the French University, beside the traditional objective devoted to the production of knowledge, has now also for objective to prepare student for an immediate employment. Analytical science is typically a branch of chemistry with this duality: research has to be developed to produce new techniques and strategies, and control laboratories need to implement equipment and methodology to get the correct information.

When the Bologna declaration has been pronounced, a new organisation has been proposed in the University of Lyon compared to the others French universities. We decided to create in Lyon a master degree devoted to analytical science. In the first year, the students learn about the major techniques whereas in the second year the students reinforce their knowledge and skills in advanced topics and apply them in real-life problem taking into account quality and economic constraints in the total analytical process.

The « Master Analyse et Contrôle » has been created in September 2004 (60 students per year with a Licence in chemistry, or biochemistry, or physics). Depending of the set of modules they chose in the final year, they will be more concerned by research, by industry and control laboratories or by forensic science.

During the conception of the master, the French chemical industry association, and the researchers of the Institute of Analytical Science have been involved to describe the different tasks they have to perform in their everyday works. So, we describe the "ideal post graduated student" in term of Knowledge, Skills, and Ability, to help teacher for final assessment.

[1] http://master-analyse-controle.univ-lyon1.fr/

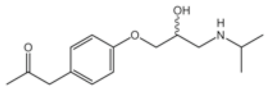
Analytical Chemistry **13**

Occurrence and Fate of β-Blockers in Sewage Treatment Plants

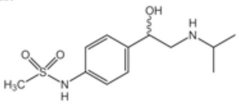
AC Alder*, M Maurer, C Schaffner, P Richle, M Böhler

Swiss Federal Institute of Aquatic Science and Technology (Eawag),
Überlandstrasse 133, CH-8600 Dübendorf, Switzerland

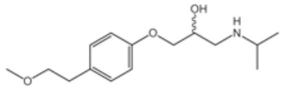
β-blockers are β-adrenoceptor antagonists drugs mainly used for treatment of cardiovascular disorders such as hypertension or cardiac arrhythmias. After intake these drugs are excreted via urine and feces as the active substance or as metabolites and reach the sewage treatment plants (STP).
In this work, the removal of four β-blockers in full-scale STPs has been investigated. Sensitive and accurate analytical methods based on LC-MS/MS have been developed to determine their concentration in the aqueous matrixes. The removal from the wastewater stream during wastewater treatment was 74–82% for atenolol, 32–36 % for sotalol, 31–43% for metoprolol and 26–33% for propranolol. In treated wastewater average concentrations were 540 ng/L for atenolol, 250 ng/L for sotalol, 130 ng/L for metoprolol and 80 ng/L for propranolol. Additionally, sorption coefficients (K_d) to sewage sludge and biodegradation rates in STPs were determined in batch reactors. Based on consumption data, human metabolism, K_d and biodegradation rates a comparison of predicted (PEC) and measured (MEC) environmental concentrations will be shown.



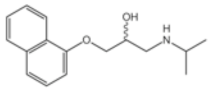
Atenolol



Sotalol



Metoprolol



Propranolol

Analytical Chemistry **14**

Desorption/Ionization on Porous Silicon for Metabolome Analysis

Andrea Amantonico and Renato Zenobi
Department of Chemistry and Applied Biosciences, ETH Zurich

Metabolome analysis is crucial for studies of drug toxicity and efficiency, and provides key information in functional genomics and for the development of efficient "cell factories". Mass spectrometry (MS) is already well established as tool for identification and quantification of metabolites. For this purpose, MS is usually coupled with separation techniques, which requires long analysis time and, in some cases, laborious sample preparation.
In this study we use desorption/ionization on porous silicon mass spectrometry (DIOS-MS) for the identification of endogenous metabolites in microorganism such as Escherichia Coli. This technique, introduced in 1999 by Siuzdak and co-workers [1], is capable of high throughput analysis and is extremely sensitive even in the absence of laborious sample clean-up procedures [2]. The low abundance of some metabolites represents a challenge in metabolome analysis, which could be met by DIOS-MS.
The physical and the chemical proprieties of the silicon active surface are critical for DIOS-MS analysis. The surfaces of commercial DIOS target plates were chemically modified with different functional groups. The performances of modified silicon surfaces were tested on a set of ten endogenous metabolites. The spectra show that amino-functionalized surfaces are particularly suitable for the detection of phosphate containing metabolites in negative mode. For the sake of comparison, the same metabolites were also analyzed with matrix-assisted laser desorption/ionization (MALDI) MS. Finally DIOS-MS and MALDI-MS were applied for the analysis of metabolites in Escherichia Coli. Different sample preparations were performed with the aim of comparing the sensitivity of these two techniques in a complex biological matrix.

[1] J. Wei, J. M. Buriak, and G. Siuzdak, *Nature* **399**, 243 (1999).
[2] S.A. Trauger, E.P. Go, Z. Shen, J.V. Apon, B.J. Compton, E.S.P. Bouvier, M.G. Finn, and G. Siuzdak, *Anal. Chem.* **76**, 4484 (2004)

Analytical Chemistry **15**

Arsenic Speciation by Gradient Anion Exchange Chromatography and High Resolution ICP MS Detection

Adrian A. Ammann

EAWAG, Swiss Federal Institute of Aquatic Science and Technology,
PO box 611, CH-8600 Dübendorf, Switzerland

Accumulating evidence on multiple toxicity aspects [1] of several arsenic species curbed down regulatory limits (WHO, US-EPA) to 10 μg/L As. Chronic toxic effects in combination with As-mobilisation [2], Arsenic is likely to pose one of the greatest threat to human health worldwide. Since years this is reality for about 50 million people in India [3], Bangladesh [4], Vietnam [5] and other places on the world [1]. Such intensified As-problems initiated many investigations to reassess the mobilisation, transformation and toxicity of even low concentrated As-species.
Common As-species cover the whole range of molecule polarities, e.g. anions, cations and, depending on the pH, neutral molecules. This and the growing number and diversity of As-compounds is a permanent challenge to ion chromatography (IC) as the over helming part of As-speciation is done by this method. A new strategy in As speciation by anion exchange chromatography is presented. It is based on the extraordinary high sensitivity of a HR ICP MS coupled to a narrow bore column with flow rates compatible to higher efficiency nebulizers for a split less direct inlet to the MS. Samples can be more diluted while analyte concentrations remain above detection limits (ng/L) less sample matrix is loaded to the column. In combination with a perfectly ICP-compatible eluent, a more robust separation and reliable As-speciation was obtained.

[1] B.K. Mandal, K.T. Suzuki, *Talanta* **2002,** *58,* 201.
[2] P.L. Smedley, D.G. Kinniburgh, Applied Geochemistry **2002,** *17,* 517.
[3] D. Chakraborti, et al., , *Talanta* **2002,** *58,* 3.
[4] J.A. Plantet et al., in S.B. Lollar (Editor), *Environmental Geochemistry* Vol. 9, Elsevier-Pergamon, Oxford, 2005, p. 17.
[5] M. Berg, et al., *Environ. Sci. Technol*. **2001,** *35,* 2621.

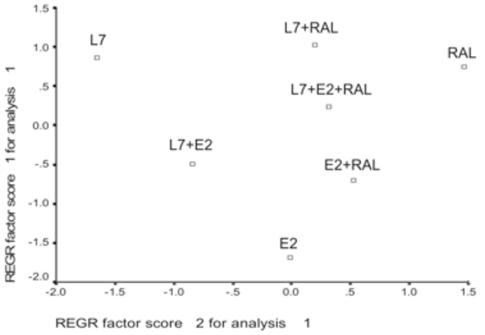
Analytical Chemistry **16**

Lipase/Esterase Activity Fingerprinting with a Substrate Cocktail

Peter Babiak, Jean-Louis Reymond

Department of Chemistry and Biochemistry, University of Berne,
Freiestrasse 3, 3012 Berne, Switzerland

Lipases and Esterases are ubiquitous enzymes useful for biotechnology and as desease markers. We recently reported an HPLC-based method for functional fingerprinting of these enzymes using a substrate cocktail [1]. Herein we report an improved version of this cocktail and its application to analyze enzymes from diverse sources. The HPLC-based fingerprint analysis is shown to be robust and reproducible. The data delivers sufficient information for useful functional identification and classification of different enzymes. For example binary and ternary mixtures of enzymes are readily identified by their fingerprints compared to pure enzymes, as shown in the figure below.



[1] J.-P. Goddard, J.-L. Reymond, *J. Am. Chem. Soc.* **2004**, *126*, 11116-11117.

Analytical Chemistry **17**

Characterization of carbon species deposited on Ni catalyst

Dimitris Bachelin[1], Rudolf Struis[1,2] and Alexander Wokaun[1]

[1] Paul Scherrer Institut (PSI), General Energy Research Department (ENE)
CH-5232 Villigen, PSI, Switzerland
[2]Swiss Federal Institute of Technology at Lausanne (EPFL) School of Architecture, Civil and Environmental Engineering (ENAC-ISTE),
Station 2, CH-1015 Lausanne, Switzerland

Carbon deposition on Ni catalyst plays an important role in catalytic processes such as the methanation process, hydrogen production or the cracking of hydrocarbons. Deposited carbon compounds can act either as reactive species (surface carbide) or as poison (polymeric carbon encapsulating the catalytic surface).

The main goal of our work is to select and use relevant analytical tools for the characterization of the carbon deposition in terms of a) reaction rate, b) carbon species identification (carbides, polymers or fibers), c) reactivity of deposited carbons (intermediate, inert or poison), and d) the location of the deposits on the catalyst (surface, bulk).

Different reaction rates (ethylene decomposition, methanation and carbidization) were investigated with thermo-gravimetric methods (TG). It was found that the size of the catalytically active nickel particles highly influences the accumulation rate of deposits. Carbidization measurements (CO at 1 atm and 265°C) supported the hypothesis that smaller particles favor the carbon deposition. The reactivity of different carbon species was studied using temperature programmed oxidation (TPO) and temperature programmed reduction (TPR). It was found that carbon alpha (C_α) synthesized by crushing pure Ni_3C was the most reactive species (the temperature of oxidation of C_α was 200-250°C). XRD was used to identify crystalline phases. Only Ni_3C and graphitic carbon were detected with XRD. In order to identify non crystalline species (polymeric carbon), Raman spectroscopic measurements are planned.

Analytical Chemistry **18**

High-mass MALDI ToF mass spectrometric analysis of cell protein extracts separated by 2-D chromatography

Claudia Bich[1], Alexandre Hainard[2], Catherine Zimmermann[2], Alexis Nazabal[1] And Renato Zenobi[1]

[1] Laboratory of Organic chemistry, HCI, ETH Zürich
[2] Laboratory of Biomedical Proteomics Research, Geneva

To analyze cell protein extracts by mass spectrometry, a separation step is required. After separation, the fractions generated are generally submitted to tryptic digestion before MS/MS analysis. Using a MALDI ToF mass spectrometer equipped with high-mass detection (Macromizer, Comet Switzerland), we have directly analyzed a large number of fractions separated by 2-dimentional liquid chromatography (ProteomLab, Beckman Coulter) without proteolysis. The specificity of the mass spectrometer used allows the detection of mixtures of intact proteins in the 5-500 kDa range.

We have performed protein profiling on a large number of fractions generated from two different samples: (i) plasma and (ii) a protein extract from staphylococcus. The standard method i.e. tryptic digestion with MS-MS analysis give more information but is extremely laborious.

First, we worked with plasma samples to evaluate the influence of albumin on the separation. In most of the fractions high-mass MS reveled the presence of albumin. In a second step, we evaluated the separation of a protein extract from staphylococcus. In most of the fractions analyzed, 2-4 proteins where detected using high-mass MALDI ToF MS. Using a concentration step prior high-mass analysis, the number of proteins detected in the fractions increased to 6-8 for the same fraction, and to 14-19 for some fractions. These experiments underline the efficiency of high-mass MALDI mass spectrometry to generate protein profiles from 2D-LC fractionations. These results will be used to determine future conditions for high-throughput protein profiling of complex cell protein extracts.

Analytical Chemistry **19**

Developments of an Analytical Method to Investigate CeO_2 Nano-Particle Uptake into Cells

Karin Birbaum[1], Ludwig Limbach[2], Wendelin Stark[2], Detlef Günther[1]

[1] Laboratory of Inorganic Chemistry, ETH Zurich, Wolfgang-Pauli Strasse 10, 8093 Zurich, Switzerland
[2] Institute for Chemical and Bioengineering, ETH Zurich, Wolfgang-Pauli Strasse 10, 8093 Zurich, Switzerland

The health risk of Nanoparticles (Np) is currently a hot research topic. Due to the large amount of industrial production CeO_2 particles, this type of Np was used to study the uptake into lung cells. To gain information about the toxicological effects of Np, all uptake studies were carried out at physiologically relevant concentrations. It was shown that simultaneously agglomeration, sedimentation and diffusion of Np are important for cell uptake[1]. The quantification of the Np-transfer into cells was carried out using solution nebulization inductively coupled plasma mass spectrometry. These studies have been carried out in suspensions (Np + Cells). Afterwards, cells have been extracted, digested and analyzed. Therefore, surface contamination could not be excluded when digesting the cell cultures. To validate that surface contamination had in insignificant influence on the quantitative description of the Np transfer into cell cultures, different washing procedures for the cells cultures were investigated and the results will be presented.

To extend our studies on Np-uptake, the sample preparation procedure (digestion) has been evaluated to be most time-consuming. Therefore, direct introduction of cells cultures into the ICP-MS was studied. Therefore, different size fractions of Np (ceria) were suspended (slurries) and introduced into the ICP-MS. The concentrations of directly introduced Np were determined using solution calibration. The 'completeness' of particle vaporization within the ICP is a prerequisite to apply such a quantification procedure. The critical size and concentration of Np for complete vaporization and ionization in the ICP was investigated and will be discussed in detail.

[1] Limbach L., *Environ. Sci. Technol.*, **2005**, *39*, 9370

Analytical Chemistry **20**

Multivariate Data Analysis of Rapid LC-TOF/MS Experiments from Arabidopsis Thaliana Stressed by Wounding

Julien Boccard[1,2], Elia Grata[1,3],Gaëtan Glauser[1,3], Pierre-Alain Carrupt[2], Jean-Luc Wolfender[3] and Serge Rudaz[1]

[1]Laboratory of Pharmaceutical Analytical Chemistry, School of Pharmaceutical Sciences, EPGL, University of Geneva, University of Lausanne,
20, Bd d'Ivoy – CH-1211 Geneva 4, Switzerland
[2]LCT-Pharmacochemistry, School of Pharmaceutical Sciences, EPGL, University of Geneva, University of Lausanne,
30, quai Ernest-Ansermet - CH-1211 Geneva 4, Switzerland
[3]Laboratory of Pharmacognosy and Phytochemistry, School of Pharmaceutical Sciences, EPGL, University of Geneva, University of Lausanne,
30, quai Ernest-Ansermet - CH-1211 Geneva 4, Switzerland

A metabolomic strategy based on a Liquid Chromatography (LC) method coupled with a high resolution time-of-flight (TOF) mass spectrometer (MS) has been developed to detect metabolic modifications occurring in Arabidopsis thaliana upon stress induction. The method was evaluated for its potential of fast discrimination between stressed (wounding by forceps) versus control Arabidopsis specimens, based on a metabolic fingerprinting survey.

Multivariate analysis was applied to handle the large amount of data generated and extract relevant information. Signal variations were filtered with an ANOVA test or an Orthogonal Signal Correction (OSC) filter to select discriminant m/z detected between plant sets. Selected ions were then processed through a data reduction procedure applied to the chromatographic information generating Total Mass Spectra (TMS) and further investigated by multivariate analysis. In a first attempt, Principal Components Analysis (PCA) was used for data treatment as a non-supervised approach. PCA demonstrated a clear clusterisation of plant specimens for experiments achieved in a short period of time. This allowed to select the highest discriminating ions given by the complete data analysis and leaded to the specific identification of discrete induced metabolites or spiked compounds.

However, when numerous days of analysis were combined, the non-supervised PCA-HCA approach gave unsatisfactory results with undefined clusters. Therefore, supervised approach as Partial Least Squares Discriminant Analysis (PLS-DA) was necessary to obtain interpretable results. PLS-DA was perfectly able to classify samples and identify important variables responsible for the obtained classification.

Analytical Chemistry **21**

Development of a mass spectrometry-based assay for the analysis and screening of endocrine disruptors

Cédric Bovet[1], Arno Wortmann[1], Sylvia Eiler[2], Florence Granger[2], Marc Ruff[2], Bertran Gerrits[3], Dino Moras[2] and Renato Zenobi[1]

[1]ETH Zurich, Zurich, Switzerland
[2]Institut de Génétique et Biologie Moléculaire et Cellulaire, Illkirch, France
[3]Functional Genomics Center Zurich, Zurich, Switzerland

During the last decades, a variety of chemicals were released into the environment in large quantities. Some of these chemicals, known as endocrine disruptors, are able to disrupt the normal function of the endocrine system by mimicking the shape of natural estrogen and induce adverse effects on wildlife and humans. We are developing a screening method based on electrospray ionization mass spectrometry (ESI MS) to quantify the estrogen receptor (ER) affinity with possible endocrine disruptors. ESI MS is a soft ionization method that allows, under suitable conditions, the detection of noncovalent complexes in the gas phase. As a model system, we are studying a triple mutant human estrogen receptor a ligand binding domain (hERα LBD) that has a ligand binding affinity similar to the wild type [1].

Under native conditions, the ESI MS data show an equilibrium between predominantly native hERα LBD homodimer and some monomer, whereas only the monomer was observed under denaturing conditions. hERα LBD was incubated with 17β-estradiol (E2), the natural hormone, and two non steroidal drugs, 4-hydroxytamoxifen (OHT) and diethlystilbestrol (DES). The complex gas phase stability was evaluated by inducing source collisions and the results fit to the relative binding affinity determined in solution. We are extending these measurements to low affinity ligands (e.g. bisphenol A) to test the limit of this screening method. We plan also to determine the dissociation constants of endocrine disruptor library based on nondenaturing ESI MS.

[1] Gangloff, M.; Ruff, M.; Eiler, S.; Duclaud, S.; Wurtz, J.M.; Moras, D., *J.* Biol. Chem., 2001, 276, 15059-15065.

Analytical Chemistry **22**

Determination of Se together with Cr and Fe in meat samples by DRC-ICPMS. Optimization and Performance

R. Brogioli[a], N. Gerber[b], B. Hattendorf[a], C. Wenk[b], D. Günther[a],

[a] ETH-Zürich, Laboratory for Inorganic Chemistry, ETH-Hönggerberg, CH-8093 Zürich, Switzerland
[b] ETH Zürich, Institute of Animal Science, ETH Zentrum, CH-8092 Zürich, Switzerland

Se, Fe and Cr are essential elements[1] and one of the main sources for human nutrition is meat[1,2]. The accurate determination of these elements is therefore important in nutrition studies. Inductively coupled plasma mass spectrometry (ICPMS) allows rapid quantitative analysis of many elements with excellent limits of detection. Determination of Se, however is problematic in conventional ICPMS due to abundant interferences from the plasma background. In order accomplish with the low concentrations in meat, a dynamic reaction cell (DRC) ICPMS was used, that allows minimizing these interferences by ion-molecule reactions with methane[3]. The great advantage of the DRC is that it allows the determination of Fe and Cr under identical operating conditions with improved signal/background ratios. The operating conditions of the dynamic reaction cell (reaction gas flow and bandpass transmission) were optimized using synthetic standard solutions. It was found that in cell-generated interferences are formed when Zn or Ca are present in the sample solution. Complete removal of these new interferences was achieved by reducing the bandpass width[4]. For validation of the analytical performance, the SRM 1577 (bovine liver) from NIST was analyzed. Good agreement with the certified concentrations indicates the suitability of this method in real world samples.

[1] D.H. Buss and H.J. Rose, *Food Chem.,* **1992,** 43, 209-212.
[2] L.H. Foster and S. Sumar, *Crit. Rev. Food Chem.* **1997,** 37, 211-228
[3] B. Hattendorf, D. Günther, *Spectrochimica Acta Part B.* **2003,** 58, 1-13.
[4] B. Hattendorf, D. Günther, *J. Anal. At. Spectron.* **2004,** 19, 600-606.

Analytical Chemistry **23**

Direct fingerprinting of human breath by EESI-TOF-MS

Huanwen Chen Arno Wortmann Renato Zenobi*

Laboratorium für Organische Chemie, ETH Hönggerberg, HCI CH-8093 Zürich, Switzerland

Rapid analysis of volatile constituents in exhaled gas provides useful insights into biochemical processes in the body, because compounds present in breath directly reflect the constituents and their relative changes in the bloodstream, thus revealing valuable information about metabolism[1]. Exhaled air, however, is rarely used for diagnostic purposes due to analytical difficulties, which include (a) low concentration of the volatile constituents, (b) condensation of volatile substances onto the wall of the breath-collecting vessel, (c) high amount of water present in exhaled air and (d) contamination from sample pretreatment.

Extractive electrospray ionization (EESI), a newly developed technique for fast mass spectrometric analysis of various samples with dirty matrices[2], is coupled directly to a Qtof-MS with minimum instrumental modification for rapid online human breath fingerprinting. Besides the intrinsic advantages of mass spectrometry such as high sensitivity and specificity, EESI-TOF-MS allows fast quantitative detection of trace analytes without sample pretreatment and facilitates ion/molecule reactions at atmospheric pressure. Results of different subjects such as heathy adults, smokers, alcohol drinkers and over-night fasted adults will be presented. Prospects of EESI-MS for breath fingerprinting and differential metabolomics will also be discussed.

[1] A. B. Lindstrom, J.D. Pleil, *Biomarkers*. **2002**, 7(3): 189-208
[2] H. W. Chen, A. Venter, R. G. Cooks, *Chemical Communications.* **2006**, *19*, 2042 - 2044

Analytical Chemistry **24**

Rhizosphere Soil Solution: Methodology for Sampling, Analysis of Organic Root Exudates and Metal Speciation

Jacynthe Dessureault-Rompré[1], Bernd Nowack[1], Rainer Schulin[1], Marie-Louise Tercier-Waeber[2], Catherine Keller[3], Jörg Luster[4]

[1]Institut of Terrestrial Ecology, ETH Zurich, Switzerland.[2]Dep.of Inorganic and Analytical Chemistry, Sciences II, University of Geneva, Switzerland.[3]Université Aix-Marseille III, France.[4]Swiss Federal Institute for Forest, Snow, and Landscape Research, Birmensdorf, Switzerland.

The goal of this study was to investigate the role of organic root exudates on metal species in the rhizosphere of hyperaccumulating Thlaspi plants in order to better understand their exceptional metal accumulating capacities. For this purpose, a modified version of the rhizobox system developed by Dieffenbach *et al.* [1] was used. In this system, a flat root system is developed which allows spatially highly-resolved sampling of the interstitial water in the rhizosphere and bulk soil by means of micro tension lysimeters. For the analysis of low-molecular weight organic acids, the samples were stabilized immediately using formaldehyde [2]. The organic acid concentrations in the samples were determined (ion chromatography) and their influence on the metal speciation studied by measuring both the dynamic fraction and the total concentrations of Cu, Pb and Cd (ICP-MS). The dynamic fraction of trace metals was monitored by square wave anodic stripping voltammetry using a gel integrated microelectrode array. This sensor has been developed and used for in situ measurements in aquatic systems [3].

[1] A. Dieffenbach, A. Göttlein, E. Matzner. (1997). *Plant Soil,* **1997***, 192*, 57

[2] J. Dessureault-Rompré, B. Nowack, R. Schulin, J. Luster. *Plant Soil*, **2006**, *in press*

[3] M.-L. Tercier-Waeber, J. Buffle, M. Koudelka-Hep, F. Graziottin. In: M. Taillefert, T.F. Rozan (Eds.), "*Environmental Electrochemistry: Analysis of Trace Element Biogeochemistry".* ACS Series No. 811, Washington DC, **2002**, pp. 16-39.

Analytical Chemistry **25**

Mass Spectrometers for the Chemical Analysis of Organic Substances (MS-CHAOS) in the Gas-and Aerosol-Phase

Josef Dommen for the MS-CHAOS Team

Laboratory of Atmospheric Chemistry, Paul Scherrer Institut, 5232 Villigen
Switzerland

Recent work has highlighted the importance of oligomeric molecules within secondary organic aerosol (SOA) [1] which may have significant implications for the physical behavior of the SOA particles in the atmosphere, as well as for their behavior in atmospheric models.

Joint experiments of seven research groups were performed at the PSI smog chamber to investigate the formation of oligomers in SOA and to test the potential of various mass spectrometric techniques in an on-line manner for that purpose. The following instruments/systems were involved: TSI Single Particle Aerosol TOF-MS (ATOF-MS from Carleton College, USA; three Aerosol Mass Spectrometers (AMS) with different ionization techniques (EI, VUV, electron attachment) and detection systems (Q-MS, V-TOF-MS, W-TOF-MS) from Aerodyne Research Inc., University of Boulder, Max Planck Institute Mainz and PSI; three Proton-transfer-reaction MS (with Q-MS, TOF-MS or coupled to GC) for gas analysis from University of Leicester, Agroscope Reckenholz and PSI; IC-MS from PSI.

The TSI-ATOFMS is capable to measure on-line large molecular masses and has the potential to derive oligomerisation-rates. Lowering the temperature of the aerosol vaporizer in the Aerodyne-AMS improves the detection capability of oligomers for the quadrupole-AMS. The V-TOF-AMS with electron impact ionization is able to measure large molecular masses because of much better detection limits compared to the quadrupole-AMS. The high resolution AMS (W-TOF) allows for observing of exact masses and following their time evolution. This provides the possibility to determine C/N/O ratios.

[1] M. Kalberer et al., Science **2004**, *303*, 1659.

Analytical Chemistry **26**

Polyamine toxins in the venom of the spider *Larinioides folium*

Silvan Eichenberger, Laurent Bigler and Stefan Bienz

University of Zurich, Winterthurerstr. 190, CH-8057 Zurich, Switzerland

The lyophilized venom of the spider *Larinioides folium* (Fam.: Araneidae) was analyzed by high-performance liquid chromatography, coupled on-line to electrospray ionization mass spectrometry (HPLC-ESI-MS) and tandem mass spectrometry (HPLC-ESI-MS/MS), respectively, as well as to UV-DAD-analysis [1]. With these combined techniques, polyamine-containing compounds were separated from the complex toxin mixtures, and the UV- and MS/MS-spectra of the separated polyamine derivatives were used to elucidate their structures. To fascilitate the structure determinations, the MS/MS-experiments were supplemented by hydrogen/deuterium (H/D) exchange HPLC-ESI-MS studies to determine the number of exchangeable protons of the investigated compounds. In addition, the venom was also fractionated by HPLC to isolate the polyamine derivatives, and the separate fractions were analyzed by high resolution mass spectrometry to confirm the molecular formulae of the suggested structures. Finally, the proposed amino acid linker of the isolated polyamine derivatives were determined by amino acid analyses. The combined analytical data, namely the UV- and MS/MS-spectra, the H/D exchange and amino acid analyses, together with the high resolution masses of the investigated compounds, resulted in the detection and characterization of several new acylpolyamines.

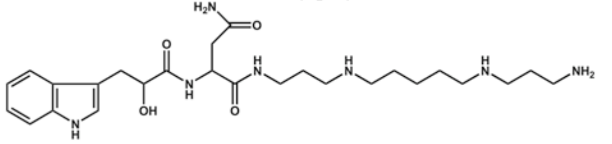


Fig: Structure of the main polyamine-containing compound in the venom of *Larinioides folium.*

[1] S. Chesnov, L. Bigler, M. Hesse, *Eur. J. Mass Spectrom.* **2002**, *8*, 1-16

Analytical Chemistry **27**

Pulsed Glow Discharge Time of Flight Mass Spectrometry Coupled to Gas Chromatography -
Expanding the Information Volume for Chemical Speciation Analysis

Daniel Fliegel[#], Katrin Fuhrer[ж], Marc Gonin[ж] and Detlef Günther[#]

[#]ETH Hönggerberg, Laboratory for inorganic Chemistry, 8093 Zürich,
Switzerland
[ж]Tofwerk AG, 3602 Thun, Switzerland

Pulsed glow discharges have been shown to be a versatile excitation an ionization source for quantitative chemical speciation analysis[1,2]. However the limitations of pulsed glow discharge TOFMS were up to now high limits of detection compared to state of the art GC-detectors as well as the acquisition and processing of large amounts of data (up to several GB/min)[3]. This allowed only the continuous monitoring of certain positions of the GD-pulse[3].

This contribution will focus on the figures of merit of a new designed GD-TOFMS[4]. Additionally the capabilities of real time mapping of the complete GD pulse during an entire GC-run will be discussed. The gain in information when acquiring data of a GC-run and GD-pulse over the entire mass range will be shown.

[1] Lewis CL, Moser MA, Dale DE, Hang W, Hassell C, King FL and Majidi V, Anal. Chem., 2003, 75, 1983
[2] Majidi V, Moser M, Lewis C, Hang W and King FL, JAAS, 2000, 15, 19
[3] Fliegel D, Waddell R, Majidi V, Günther D and Lewis CL, Anal. Chem., 2005, 77, 1847
[4] Fliegel D, Fuhrer K, Gonin M and Günther D, 2006, ABC, accepted

---

Analytical Chemistry **28**

Quantification of Major Elements in Phase Change Materials using LA-ICP-MS

Christian Frei, Detlef Günther

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

Phase Change materials are based on the reversible transition between a crystalline and an amorphous state. Phase Change materials are important in many applications, especially the AgInSbTe alloy is widely-used in commercial rewritable optical data storage devices such as CD-RW and DVD-RW. [1]

The determination of stoichiometry within these materials is an important task in industrial quality control. In LA-ICP-MS minimum sample preparation is required, which favours this technique for such analysis.

In this study, AgInSbTe samples with different stoichiometry were quantified for major elements using an in-house AgInSbTe standard material as an external standard. The experiments were carried out using an 193nm ArF Excimer laser-system in combination with an Agilent 7500ce quadrupole ICP-MS.

The ablation behaviour of these materials was studied and quantification capabilities using different ablation modes were compared. Precision and accuracy achievable for these types of samples will be discussed in detail.

[1] W. K. Njoroge, H. Dieker, M. Wuttig: *J. Appl. Phys.* **2004**, 96, 2624

Analytical Chemistry **29**

Online IC-MS Measurements Of Organic Acids in Aerosols and the Gas-Phase During Ambient and Wood Stove Fire Studies

K. Gäggeler, J. Dommen, A.S.H. Prevot, U. Baltensperger

Paul Scherrer Institut, 5232 Villigen, Switzerland

The composition of aerosols from wood burnings is not yet identified well. Ion chromatography coupled to a mass spectrometer (IC-MS) was used to identify organic acids and inorganic anions. Sampling of ambient air took place in Roveredo, Switzerland, a village in a mountain valley in December 2006. First results indicate that wood burning is the most important source of aerosols in this region. Next to this campaign, laboratory measurements at a wood stove with several types of wood took place.

Air was aspired through the wet effluent diffusion denuder/aerosol collector (WEDD/AC) for sampling water-soluble gas-phase and aerosol compounds and then analyzed using IC-MS. The MS (MSQ from Dionex) has a single quadrupole mass detector and uses the atmospheric pressure ionization (API) technique, which operates using electrospray ionization (ESI).

In the furnace study high amounts of monoacids were found. Organic anions detected were the monoacids lactic, acetic, glyoxylic, formic, pyruvic and valeric acid, the mass-to-charge ratios 106 and 132, and the dicaboxylic acids maleic and oxalic acid as well as the inorganic anions chloride, sulfate, nitrite, nitrate.

The data from ambient measurements show small amounts of monoacids and higher concentrations of di- or tricarboxylic acids. A diurnal cycle was observed, showing highest concentrations between lunch and midnight in both gas- and aerosol-phase. This data will be compared with other measurements performed simultaneously by Aerosol mass spectrometry (AMS), PTR-MS and recordings of EC/OC, CO and NO_x.

Analytical Chemistry **30**

Search of Defence-Induced Compounds in *Arabidopsis thaliana* by UPLC-TOF-MS and CAP-NMR of a Lesion Mimic Mutant acd2-2

G. Glauser[a,b], E. Grata[a,b], S. Rudaz[b], E. Farmer[c] and J.L. Wolfender[a]

[a]LPP, [b]LCAP, School of Pharmaceutical Sciences, University of Geneva, University of Lausanne, 30 quai Ernest-Ansermet, CH-1211 Geneva 4, [c]Département de Biologie Moleculaire Végétale, UNIL, BB CH-1015 Lausanne

Different wound-inducible biomarkers which might play a role in defence signalling were localised by a LC-MS metabolomic approach where control and wounded specimens of *A. thaliana* were compared. These compounds, mainly oxylipins [1], were well separated on high peak capacity UPLC-TOF-MS columns using long gradients. The characterisation of the unknown induced compounds was however found to be very challenging because of their minute amounts (ng-µg/g of fresh leaves) and the convoluted nature of the extracts.

To obtain preliminary information on the nature of these induced metabolites, a mutant acd2-2 (accelerated cell death), which presents spontaneous lesions and is known to overproduce oxylipins, was investigated. Compared to the wounded wild type specimens, a clear increase of the oxylipin LC-peaks was observed in this mutant. Based on these observations, an efficient selection of relevant induced metabolites was obtained. Their direct LC-MS triggered fractionation from crude extracts was however not possible at a semi-preparative level because of co-elution with constitutive metabolites present in large amounts. In this respect, sample preparation procedures based on enrichment by preparative scale SPE and acid base LLE were devised, followed by a separation on high resolution analytical columns. This approach enabled the structural assignment of some of the low molecular mass regulators of interest at the microgram level by capillary LC-NMR.

Acknowledgements: SNSF is thanked (grant 205320-107735 to JLW).

[1] E.E. Farmer, E. Almeras, V. Krishnamurthy, *Curr. Opin. Plant Biol.* **2003**, *6*, 1.

Analytical Chemistry **31**

SI-traceable Mass Spectrometric Analysis of Gas Mixtures

Hans-Peter Haerri and Daniel Schwaller

Swiss Federal Office of Metrology (METAS)
Lindenweg 50, 3003 Bern-Wabern, Switzerland

The major use of standard gas mixtures with amount of substance fractions in the ppb range is the calibration of ground level gas monitors of atmospheric monitoring agencies [1]. The purities of the analytes, the dilution gases and the zero gases are important quantities for their uncertainties [2].

The method based on a commercial quadrupol mass spectrometer (MS) with ion-molecule reaction ionization along with the instrumental modifications have been described in previous publications [3]. The reproducibility of results has largely improved by rigorously controlling the measurement parameters, by using longer sampling times, external data analysis and a gas dilution system for setting the amount of substance fractions. The implemented computer-controlled gas selection unit [4] in combination with measurement procedures based on LabView and TestStand sequences [5] (both National Instruments) allow a fully automated operation of the MS and all peripherals.

The measurement procedure, the data analysis and uncertainty calculation for a NO in N_2 mixture with traces of NO_2, the validation with a number of air pollutants and the cleaning efficiency of a zero air generator will be presented. ppb detection limits for a number of analytes are reached.

[1] D.W. Zickert, Actes de conférence, 11ème Congrès International de Métrologie 2003, Toulon, France.
[2] H.-P. Haerri, EUROMET Workshop on purity analysis in pure and balance gases, LNE Paris-France, 8./9.9.2003.
[3] H.-P. Haerri, metINFO 10 (3), 2003.
http://www.metas.ch/de/metINFO/2003/metINFO03_3.html
[4] D. Kühni, Inetronic AG, Zollstrasse 10, 3436 Zollbrück.
[5] Iset GmbH, Bahnweg 2, CH-3177 Laupen, heinz.herren@iset.ch.

Analytical Chemistry **32**

Conservation Research and Analytical Chemistry at the Swiss National Museum

Vera Hubert

Swiss National Museum, Laboratory for Conservation and Research,
Hardturmstrasse 181, CH 8005 Zürich, Switzerland

The Laboratory for Conservation and Research of the Swiss National Museum performs chemical and physical analyses on valuable museum objects not allowing any visible damage. Analytical questions of the conservators of the Swiss National Museum, other museums and archaeological institutions are addressed with various techniques like Fourier-transform infrared spectrometry (FT-IR), Raman micro spectrometry, micro X-ray fluorescence spectrometry (µ-XRF) and graphite furnace atomic absorption spectrometry (GFAAS). Moreover, the laboratory collaborates with institutions like the Paul Scherrer Institute (PSI), the Laboratory of Inorganic Chemistry (ETHZ), the Institute for Mineralogy and Petrography (ETHZ) and the Institute of Isotope Geochemistry and Mineral Resources (ETHZ) in order to apply other methods for solving archaeometric problems.

The European network COST G8 ”Non-destructive investigation of museum objects” enables projects for specialised studies. Within this framework, the Laboratory for Conservation Research has established interdisciplinary projects with its collaboration partners. Examples for these projects are investigations of Roman bronze statuettes, of Neolithic copper objects and of silver artefacts from the Giubiasco graveyards. The analyses have been performed by neutron radiography and tomography, AAS, µ-XRF, laser ablation ICP-MS and electron microprobe analysis. Furthermore, some of the objects have been investigated by Pb isotope analysis.

Analytical Chemistry **33**

A new Approach for Quick ^{13}C NMR Relaxation Rate Measurements in Small Molecules

Bruno Vitorge, **Fehler! Verweisquelle konnte nicht gefunden werden.**

Department of Organic Chemistry, University of Geneva,
30 Quai E. Ansermet, 1211 Geneva-4, Switzerland

^{13}C NMR relaxation rates provide important information about molecular sizes and dynamic behaviors. The measurements of T_1 and T_2 can be made using 1D spectra, but 2D heteronuclear experiments are much more sensitive and provide much higher precision and accuracy. The problem with 2D experiments is that acquisition times of the series of spectra may be impractical when one needs to resolve clusters of carbon signals. Computer optimized spectral aliasing, a general method to decrease the acquisition times of heteronuclear experiments by one to two orders of magnitude,[1] has already been applied to the diffusion measurements of individual species in complex mixtures.[2] The poster will show that similar gains in acquisition times can be reached in relaxation measurements.

[1] D. Jeannerat, D. Ronan, Y. Baudry, A. Pinto, J.-P. Saulnier and S. Matile, *Helv. Chim. Acta*, **2004**, *87*, 2190
[2] B. Vitorge, D. Jeannerat, *Anal. Chem.* in press.

Analytical Chemistry **34**

Phenolic Substances with Endocrine Disruptor Potential in the Aquatic Environment: a Mass Flow Analysis

Niels Jonkers, Hans-Peter E. Kohler, Walter Giger

Swiss Federal Institute of Aquatic Science and Technology (Eawag)
Ueberlandstrasse 133, CH-8600 Dübendorf, Switzerland

A number of phenolic compounds have the potential to disturb the hormone system of organisms and are called endocrine disrupting compounds (EDCs). The fate of many of these substances in the aquatic environment is largely unknown.

This study focused on the behaviour in wastewater and surface waters of several phenolic EDCs, including parabens, alkylphenolic compounds, and bisphenol-A. Analytical procedures using SPE and LC-MSMS techniques were optimized, and applied to samples of influents and effluents of wastewater treatment plants (WWTPs) discharging into the Glatt River (Switzerland), as well as to river water samples.

Concentrations in influents were in the low µg/L range for most analytes. Removal of parabens in the WWTPs was mostly >95%, while for the alkylphenolics, removal was lower, and significant formation of metabolites was observed. In effluents, concentrations were highest for the nonylphenoxy acetic acids and nonylphenols. Concentrations in river water were in the high ng/L range for alkylphenolic compounds and in the low ng/L range for bisphenol-A and parabens.

In a mass flow analysis, the input mass flows of the EDCs were compared with the actual mass flows at the end of the river. Significant removal in the Glatt river (up to 70%) was observed only for the parabens.

Analytical Chemistry **35**

Investigation of Hopanoid Biomarkers in Lake Sediments

Gamze Kavran Belin, Frédéric Houot, Fazil O. Gülaçar

Laboratory of Mass Spectrometry, University of Geneva, Science-I
Boulevard d'Yvoy, 1211, Geneva, Switzerland

Hopanoids, whose primary function is to improve plasma membrane fluidity in prokaryotes, are a class of pentacyclic compounds which are abundant in ancient sediments and oils. The formation of hopanoids in geological materials shows the importance of bacterial lipid contributions to sediment and petroleum samples [1]. Moreover, hopanoids are not easily degraded since they have a very stable skeleton structure resisting to degradation. Therefore, more attention has been focused on their usage as biological markers. These biomarkers are mainly derived from bacteriohopanepolyols (Fig.1) which occur especially in bacteria.

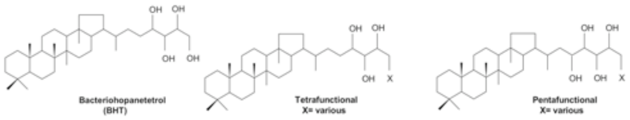


Figure 1. The structure of bacteriohopanpolyols.

In this work, GC-MS and HPLC-MS analyses of hopanoid biomarkers and biological precursors in oxic and anoxic sediment samples from Lake Cadagno and Voua de la Motte are presented and discussed to estimate their possible different bacterial contributions.

[1] Ourisson, G., Rohmer, M., Poralla, K., *Annu. Rev. Microbiol.* **1987**, *41*, 301-333.

Analytical Chemistry **36**

Elemental Analysis of nm-sized Particles and Segregations

F. Krumeich and R. Nesper

Laboratory of Inorganic Chemistry, ETH Zurich, 8093 Zurich, Switzerland

Scanning transmission electron microscopy performed with a high-angle annular dark field detector (HAADF-STEM) detects incoherently scattered electrons only and thereby provides high-resolution information about the elemental distribution (Z contrast imaging) [1]. Furthermore, the electron beam can be set onto nm-sized specimen areas, which allows one to record X-ray and electron energy loss spectra at these selected spots. This technique has been applied for the characterization of bimetallic catalysts (e.g., Pd-Pt on alumina, figure 1 [2]) and of segregations in oxides [3].

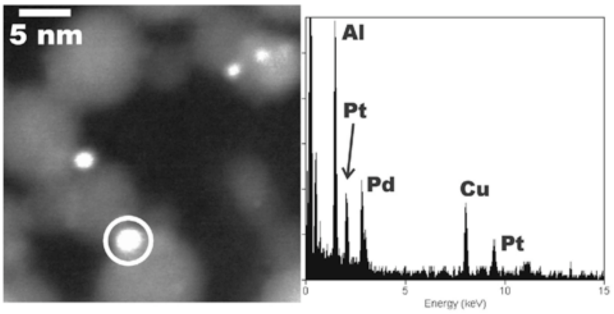


Figure 1. HAADF-STEM image (left) of metal particles (bright spots) on alumina. The presence of Pd and Pt peaks in the EDX spectrum (right) of the encircled particle indicates alloy formation [2].

[1] S. J. Pennycook, *Ultramicroscopy* **30** (1989) 58.
[2] R. Strobel, J.-D. Grunwaldt, A. Camenzind, S. E. Pratsinis, A. Baiker, *Catal. Lett.* **104** (2005) 9.
[3] F. Krumeich and R. Nesper, *J. Solid State Chem.* **179** (2006) 1658.

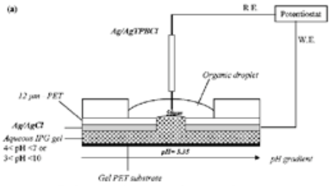
Analytical Chemistry **37**

Immobilized pH Gradient Gel Cell to Study the pH Dependence of Drug Lipophilicity

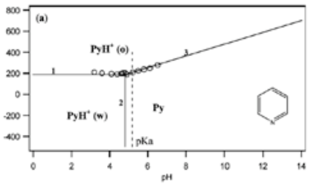
Hoang-Trang Lam[1], Christophe Roussel[1], Carlos M. Pereira[2], Pierre-Alain Carrupt[3], Hubert H. Girault[1]*

[1] Laboratoire d'Electrochimie Physique et Analytique, Ecole Polytechnique Fédérale de Lausanne, Station 6, CH-1015 Lausanne (Suisse)
[2] Departamento de Quimica, Faculdade de Ciências da Universidade do Porto, 4169-007 Porto (Portugal)
[3] Unité de Chimie Pharmaceutique, Université de Genève, CH-1211 Genève (Suisse)

We report herein an original setup to study the pH dependence of standard ion-transfer potentials at Water/NPOE (nitrophenyloctylether) interface [1]. In this setup (scheme 1), a commercial Immobilized pH Gradient gel, acting as aqueous phase, is used to access to the partition diagram of several drugs (Scheme 2) by electrochemistry. The system allows a rapid evaluation of the partition coefficient of different drugs, the value of which is used to find out the potential drug activity in pharmaceutics.



Scheme 1: experimental setup



Scheme 2: partition diagram of Pyridine

This original approach provides a high-throughput method dedicated to pharmaceutical screening.

[1] H.-T. Lam, C. M. Pereira, C. Roussel, P.-A. Carrupt, H. H. Girault, *Anal. Chem.* **2006**, *78*, 1503-1508.

Analytical Chemistry **39**

Evaluation of the Influence of Protein Precipitation Prior to on-Line SPE-LC-API/MS Procedures Using Multivariate Data Analysis

Ivano Marchi, Serge Rudaz, Jean-Luc Veuthey

Laboratory of Analytical Pharmaceutical Chemistry, School of Pharmaceutical sciences, University of Geneva, University of Lausanne, Bd d'Yvoy 20, 1211 Geneva 4, Switzerland

Matrix effect on mass spectrometry (MS) response was investigated with atmospheric pressure ionization (API) sources after on-line solid-phase extraction (SPE) of human plasma. A post-column infusion system was used to measure the MS signal alterations of eight analytes chosen as model compounds. On-line SPE was evaluated with one restricted access material (RAM), two large particle supports (LPS) and one monolith. A sample pretreatment (protein precipitation (PP)) and a direct injection (dilution) were tested. Principal component analysis (PCA) was performed to simplify data presentation and interpretation. For all extraction materials and ionization sources, PP was found mandatory for significantly reducing signal modification due to endogenous components eluted from the extraction support. Regarding sensitivity towards matrix effects after PP, APPI was globally the least sensitive ionization mode while ESI was the most sensitive.

Analytical Chemistry **38**

Study on the Formation of Furan Using the Carbon Module Labeling (CAMOLA) Technique

Anita Limacher[1], Josef Kerler[2], Beatrice Conde-Petit[1], Imre Blank[2]

[1]ETH Zurich, CH-8092 Zurich, Switzerland
[2]Nestlé Product Technology Center Orbe, CH-1350 Orbe, Switzerland

Furan has recently been reported to occur in certain food products that are subject to thermal treatment, especially canned and jarred products [1]. This has received attention, as furan is considered as a possibly hazardous compound, classified by the IARC as "possibly carcinogenic to humans" (group 2B) [2]. Previous model system studies showed furan to be formed from ascorbic acid, sugars, Maillard reaction systems, and polyunsaturated fatty acids [3][4]. We report here on mechanistic studies with the focus on elucidating the formation pathways of furan from different precursors. Thereby, the Carbon Module Labeling (CAMOLA) technique [5] was applied to investigate whether the carbohydrate skeleton remains intact upon furan formation or how furan is formed by the recombination of two carbon moieties. The technique allowed suggesting new formation pathways of furan in model systems as well as in foods.

[1] U.S. Food and Drug Administration (May 7, 2004; updated June 7, 2004), http://www.cfsan.fda.gov/dms/furandat.html.
[2] International Agency for Research on Cancer. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans; Lyon, France. **1995**, *63*, 3194 – 3407.
[3] C. Perez Locas, V. A. Yaylayan, *J. Agric. Food Chem.* **2004**, *42*, 6830-6836.
[4] Märk, J.; Pollien, P.; Lindinger, C.; Blank, I.; Märk, T. *J. Agric. Food Chem.* **2006**, *54*, 2786-2793.
[5] Schieberle, P. *Ann. N.Y. Acad. Sci.* **2005**, *1043*, 236-248.

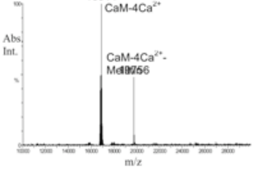
Analytical Chemistry **40**

Comparison of Dissociation Constants of a Model Protein-Ligand Complex Determined using Standard and Mass Spectrometric Techniques

Sonal Mathur, Michael Scott, Renato Zenobi*

Department of Chemistry and Applied Biosciences, Wolfgang-Pauli-Strasse 10, ETH Zurich, CH-8093 Zurich, Switzerland

Soft ionization mass spectrometry has been used for studying solution-phase noncovalent interactions and their binding affinities for a number of years [1]. The aim of this project is to draw a comparison between mass spectrometric techniques (ESI, nanoESI, MALDI-SUPREX), and standard methods like surface plasmon resonance (SPR) and circular dichroism (CD) to elucidate *pros* and *cons* of each technique in terms of their feasibility for the determination of binding constants of protein-ligand complexes. The binding partners selected as models for this study are calmodulin and melittin, which form a tight binding complex (1:1) with a dissociation constant of 3 nM in the presence of Ca^{2+} [2].



Preliminary results obtained from ESI-MS (K_d = 4 nM), a representative spectrum is shown above, and SPR (K_d = 6 nM) are in good agreement with the reported K_d value. The affinity determined by MALDI-SUPREX was 1.2 µM. Experiments with other techniques are underway.

[1] J. M. Daniel, S. D. Friess, S. Rajagopalan, S. Wendt, R. Zenobi, *Int. J. Mass Spectrom.* **2002**, *216*, 1.
[2] M. Comte, Y. Maulet, J. A. Cox, *Biochem. J.* **1983**, *209*, 269.

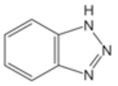
Analytical Chemistry **41**

Elimination of Benzotriazoles in Sewage Treatment

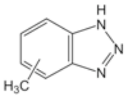
Thomas Hinterbuchner, Christa S McArdell, Eva Molnar,
Christian Schaffner, Juliane Hollender, Walter Giger,
Hans-Peter E Kohler

Swiss Federal Institute of Aquatic Science and Technology (Eawag),
Überlandstrasse 133, CH-8600 Dübendorf, Switzerland

The complexing agents benzotriazole and tolyltriazole are not only widely used as anticorrosives (e.g. in engine coolants or in antifreezing liquids), but also for so-called silver protection in dish washing liquids. Concentrations of benzotriazole found in mechanically and biologically treated municipal wastewater effluents ranged from 1 to 100 µg/L [1]. It is also detected in surface water and even in groundwater [2]. Benzotriazoles are said to be persistent to biodegradation, but in different sewage treatment plants the elimination of benzotriazole was found to vary from 0 to 50%. Natural and sterilized sewage sludge was spiked with benzotriazoles and monitored over time to elucidate biodegradation and sorption processes. Sorption was found to be the main elimination process occurring in sewage treatment plants.



1H-benzotriazole



4- and 5-methyl-1H-benzotriazole (tolyltriazole)

[1] Voutsa D, Hartmann P, Schaffner C, Giger W 2006. Alkylphenols, Bisphenol A and Benzotriazoles in the Glatt River, Switzerland. *Env. Sci. Poll. Res.* **2006**, *13*, in press.
[2] Blüm W, McArdell CS, Hoehn E, Schaubhut R, Labhart W, Bertschi S. Organische Spurenstoffe im Grundwasser des Limmattales - Ergebnisse der Untersuchungskampagne 2004. AWEL Bericht, **2005**. (http://www.bd.zh.ch > Medienforum, Dossier „Mikroverunreinigungen")

Analytical Chemistry **42**

The Standard Deviation and the Standard Deviation of the Mean: How Do I Choose?

Veronika R. Meyer

EMPA Materials Science and Research, Lerchenfeldstrasse 5, 9000 St. Gallen

In statistics, a property for the description of normally distributed values is the standard deviation. It exists in two types: the standard deviation $s(x)$ and the standard deviation of the mean $s(\bar{x})$, the latter being a smaller number than the former:

$$s(x) = \sqrt{\frac{1}{(n-1)}\sum_{i=1}^{n}(x_i - \bar{x})^2} \qquad s(\bar{x}) = \frac{s(x)}{\sqrt{n}} = \sqrt{\frac{1}{n(n-1)}\sum_{i=1}^{n}(x_i - \bar{x})^2}$$

When it comes to the calculation of measurement uncertainties, there is a temptation to use $s(\bar{x})$ because it can lower the resulting number $u_c(x)$. However, the standard deviation of the mean must only be used if a well-defined object or a well-defined process, used for the investigation of a well-defined object, is studied. For most analyses, it is the standard deviation $s(x)$ which appears in the measurement uncertainty equation. Especially, repeatabilities and recoveries need to be described by $s(x)$, irrespective of how many times they have been determined.

Analytical Chemistry **43**

Environmental Occurrence and Behaviour of Macrolide and Sulfonamide Antibiotics from Hospital and Municipal Wastewaters to Rivers and Groundwaters

Eva Molnar, Alfredo C. Alder, Christa S. McArdell, Walter Giger*

Eawag, Swiss Federal Institute of Aquatic Science and Technology, 8600 Dübendorf, Switzerland, giger@eawag.ch

Human used pharmaceuticals, including antimicrobial agents, reach the aquatic environment mainly via excretion and after passing through sewage treatment. We report here on two topics:
(A) Hospital wastewaters in Switzerland were characterized which can act as point sources for the input of pharmaceuticals into the aquatic environment. The antibiotic levels in the hospital wastewaters were generally one to two orders of magnitude higher than in the treated wastewater effluents. For example, the concentration of the macrolide antibiotic clarithromycin varied from 0.16 to 29 µg/L and in the treated wastewater effluents it ranged from 0.06 to 0.52 µg/L.
(B) Fate studies were performed in the Limmat Valley watershed in order to study inputs from the municipal wastewater treatment plant of Zurich-Werdhoelzli, to evaluate the fate of macrolides and sulfonamides in the Limmat River as well as to assess their behaviour during bank filtration into groundwater. Sulfamethoxazole could be detected in measurable concentrations (maximum 19 ng/L) in the groundwater. Only about 30% is eliminated during bank filtration.

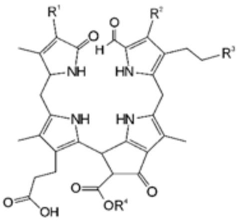
Analytical Chemistry **44**

Identification and Structural Characterization of Chlorophyll Breakdown Products in Senescent Plants using NanoLC/ESI-MS

Thomas Müller, Simone Moser, Kathrin Breuker and Bernhard Kräutler

Institute of Organic Chemistry and Center for Molecular Biosciences, Innrain 52a, A-6020 Innsbruck, Austria

Chlorophyll breakdown is the visible symptom of leaf senescence, a form of programmed cell death in plants. It is estimated that every year more than one billion tons of chlorophyll are degraded on earth. This is now known to occur in a thoroughly controlled and (basically) common path [1,2].



Basic structure of "non-fluorescent" chlorophyll catabolites (NCCs) with various substituents R1-R4 [2,3].

Most of these tetrapyrrolic compounds are very sensitive to light and air exposure, and show poor stability in crude plant extracts. Here we developed a rapid and highly efficient LC-MS method to screen plant extracts for chlorophyll catabolites, and gain structural information by analysis of characteristic fragmentation patterns.

[1] Kräutler, B., Jaun, B., Bortlik, K., Schellenberg, M., Matile, P. *Angew. Chem., Int. Ed. Engl.* **1991**, 30, 1315-1318.
[2] Müller T., Moser S., Ongania K.H., Pruzinska A., Hörtensteiner S., Kräutler B. *ChemBioChem* **2006**, 7, 40-42.
[3] Berghold, J., Müller, T., Ulrich, M., Hörtensteiner, S., Kräutler, B. *Chemical Monthly* **2006**, in Press.

Analytical Chemistry **45**

New trends in fast LC

Dao T.-T. Nguyen, Davy Guillarme, Serge Rudaz, Jean-Luc Veuthey

Laboratory of Analytical Pharmaceutical Chemistry, School of Pharmaceutical Sciences, University of Geneva, University of Lausanne, 20 Bd. d'Yvoy - 1211 Geneva 4 – Switzerland

Pharmaceutical industry has always been interested by new technologies for increasing productivity in a shorter time. Fast liquid chromatography (LC) starts to be widely used in routine analytical work, method development, process monitoring and quality control.

In LC, there are different approaches to reduce analysis time in order to obtain fast or ultra-fast methods with cycle times less than 5 or 1 min, respectively. Several strategies exist such as the reduction of column length (≤ 50 mm), increase of mobile phase flow rate, reduction of particle size (≤ 2 µm), work at high pressure (≤ 1000 bar), use of monolithic supports or use of high temperature (≤ 200°C). These approaches are not equivalent and it is not obvious to decrease analysis time by maintaining good peaks resolution. Therefore, in most of the cases, combination of several strategies is used.

This study presents the different techniques and compares them to conventional LC, with theoretical and experimental results. Advantages and drawbacks (resolution, peak capacity, instrumentation, etc.) of these different approaches are discussed in this work.

Analytical Chemistry **46**

Nanoparticle-based pseudostationary phases in CE/CEC

Christian Nilsson[1, 2], Staffan Birnbaum[2], Staffan Nilsson[1]

[1]Pure and Applied Biochemistry, Center for Chemistry and Chemical Engineering, Lund University, P.O. Box 124, 221 00 Lund, Sweden.
[2]Biopharmaceuticals Support QC, Manufacturing Development, Novo Nordisk A/S, Denmark

An alternative way to perform capillary electrochromatography (CEC), compared to traditional formats, is to use a pseudostationary phase (PSP), *i.e.* an interaction phase moving with the mobile phase[1]. PSPs benefit from one-time use, avoiding carry-over effects associated with stationary phase contamination. However, carry-over effects associated with capillary wall adsorption are still present. Dextran-coated nanoparticles have been used as PSP for highly efficient CEC separations (plate numbers up to 700 000 /m) of neutral analytes[2]. The nanoparticles, with an average diameter of 600 nm, were prepared by polymerization of methacrylic acid (MAA) and trimethylolpropane trimethacrylate (TRIM), which in turn were coated with dextran. The dextran coating enables stable nanoparticle suspensions to be formed in hydrophilic electrolytes and benefits from excellent protein compatibility. When proteins are analyzed in capillary electrophoresis (CE) or CEC, adsorption to the fused silica capillary wall and stationary phase (in CEC) has to be considered. The dextran coated nanoparticles role to suppress non-coated capillary wall adsorption of the proteins has been shown. An interesting application is protein separation by PSP-CEC, where the nanoparticles have an ambiguous role as interaction phase and additive to avoid protein adsorption.

[1] Nilsson, C.; Nilsson, S. *Electrophoresis* **2006**, *27*, 76-83
[2] Nilsson, C.; Viberg P.; Spegel, P.; Jornten-Karlsson, M.; Petersson, P.; Nilsson, S. *Anal. Chem.* **(accepted)**

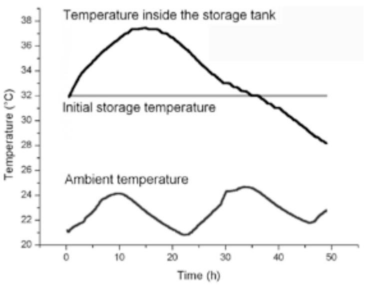
Analytical Chemistry **47**

Determination of the oxidation states of iron present in the dried sewage sludge

Martine Poffet [a)], Kurt Käser [b)], Titus A. Jenny [a) *]

a) University of Fribourg, Chemistry Department
b) University of Applied Sciences, Department of Industrial Technologies
CH-1700 Fribourg, Switzerland

A main portion of the dried sewage sludge produced in Switzerland is incinerated in the cement industries. This important waste is reduced to ashes and its energetic contents are used as an alternative fuel. During the storage, significant temperature increases occur what could induce a thermal runaway. Labscale storage experiments have been processed and spontaneous temperature increases were observed directly at the beginning of the storage.



In the analyzed dried sewage sludge, iron represents around 10 % of the total dried mass. A Fe^{2+} compound was determined in the crystalline phase as vivianite: $Fe_3(PO_4)_2 \cdot 8\ H_2O$.
This work studies the evolution of the Fe^{2+} / Fe^{3+} ratio during storage of the dried sewage sludge. The oxidation of iron II phosphate could be correlated to the heat release observed in the storage tanks (see Figure).

Analytical Chemistry **48**

Differentiated Quantification of Bile Acids with LC-MS/MS

Ines Burkard, Arnold von Eckardstein, Katharina M. Rentsch

University Hospital Zürich, Rämistrasse 100. 8091 Zürich, Switzerland

Determination of quantitative changes in the pattern of serum bile acids is important for the monitoring of diseases affecting bile acid metabolism. A sensitive and specific HPLC–MS/MS method has been developed for the differentiated quantification of unconjugated as well as glycine- and taurine-conjugated cholic, chenodeoxycholic, deoxycholic, ursodeoxycholic and lithocholic acid in serum samples. After solid-phase extraction and reversed-phase HPLC separation, detection of the conjugated bile acids was performed using electrospray ionization (ESI)-MS/MS and selected reaction monitoring mode, whereas unconjugated bile acids were determined by ESI-MS and selected ion monitoring mode.
The influence of daytime, food intake and menstrual cycle on different bile acid concentrations was investigated in a small healthy collective. Moreover, reference intervals for fifteen different bile acids were established in 100 healthy volunteers. Bile acids were found to undergo diurnal changes, whereby only the conjugated derivatives increased after food intake. In comparison to initial fasting-state levels, the maximal postprandial increase for glycine and taurine conjugates ranged between 120% and 1800%. In contrast to conjugated bile acids, unconjugated bile acid concentrations in fasting morning blood samples showed large variations – leading to the hypothesis that the earlier in the morning the blood sampling the higher the concentrations of unconjugated bile acids. Furthermore, upper reference limits – representing the 95th percentile – were determined for all fifteen different bile acids. Significant differences in bile acid levels between different age groups could not be observed.

[1] Burkard I, von Eckardstein A, Rentsch KM, *J Chromatog.r B* **2005**, *826*, 147.

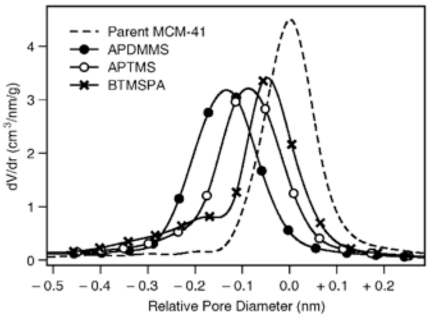
Analytical Chemistry **49**

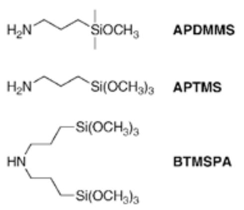
Distribution of Grafted Aminopropylalkoxysilanes on the Surface of Mesoporous Silica MCM-41

Hanna Salmio and Dominik Brühwiler*

Institute of Inorganic Chemistry, University of Zürich
Winterthurerstrasse 190, CH-8057 Zürich, Switzerland

Aminopropylalkoxysilanes (APAS) are among the most widely used reagents for the functionalization of silica surfaces [1]. The distribution of the amino groups on MCM-41 after submonolayer deposition of APAS from anhydrous liquid phase was probed by fluorescein labeling in conjunction with luminescence spectroscopy and pore size analysis. Monoalkoxysilanes generally distributed more uniformly than monopodal and bipodal trialkoxysilanes. Furthermore, the outcome of the grafting reaction depended on the nature of the amino group, with secondary amines grafting more efficiently than their primary analogues.





[1] K. C. Vrancken, K. Possemiers, P. Van Der Voort, E. F. Vansant, *Colloids Surfaces A* **1995**, *98*, 235.

Analytical Chemistry **50**

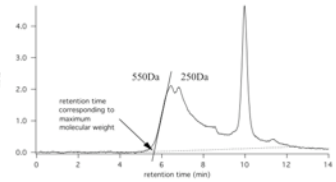
Seasonal variation and functional groups analysis of high molecular weight compounds in the water-soluble fraction of organic urban aerosols

Vera Samburova, Renato Zenobi and Markus Kalberer
Department of Chemistry and Applied Biosciences, ETH Zurich, 8093 Zurich, Switzerland

Atmospheric aerosols contain a significant fraction of organic components, up to 50% in mass, but only 10-20% of the organic mass can be resolved on a molecular level. In recent years, high molecular weight compounds have received considerable attention as components of atmospheric organic aerosols and cloud water constituents. These compounds have characteristics similar to those of humic substances, especially their UV and fluorescence spectra. They are therefore often named humic-like substances (HULIS).

Only little is known about the chemical properties of HULIS. Mass spectrometry analyses showed that this high molecular weight fraction consists of hundreds of compounds (e.g., Samburova et al., 2005a). The distribution and quantification of different functional groups allows for a characterization of this compound class. In this study aerosol samples were analyzed for water-soluble organic compounds with high molecular weight using size exclusion chromatography (SEC), laser desorption/ionization mass spectrometry (LDI-MS), Fourier transform infrared spectroscopy (FTIR), acid titration and proton nuclear magnetic resonance (^{1}H-NMR).

The molecular weight distribution of HULIS was determined by two different methods, SEC and LDI-MS. The two HULIS peaks with the highest intensity in the SEC chromatogram correspond to 550 and 250 Da, respectively (Figure 1), comparing well with the LDI-MS measurements showing the most intense peaks between 150-500 Da. The maximum molecular weight of HULIS, as determined with SEC (see Figure 1), is significantly higher in the winter (between 2200 and 3300 Da) than in the summer (1300 and 2400 Da). For the summer samples, positive correlations of the maximum molecular weight with temperature and ozone concentrations are visible. In the winter these correlations are not found, indicating that different sources and processes are involved in the HULIS formation in winter and summer, respectively (Samburova *et al*., 2005b).



[1] Samburova, V., et al., (2005a), Characterization of high molecular weight compounds in urban atmospheric particles, *Atmos. Chem. Phys., 5*, 2163-2170.
[2] Samburova, V., et al. (2005b), Seasonal variation of high-molecular-weight compounds in the water-soluble fraction of organic urban aerosols, *J. Geophys. Res.,* 110, D23210

Analytical Chemistry **51**

Benzotriazoles in Municipal Wastewaters and in the Glatt River, Switzerland

Christian Schaffner, Hans-Peter E. Kohler, Walter Giger*

Eawag, Swiss Federal Institute of Aquatic Science and Technology,
8600 Dübendorf, Switzerland, giger@eawag.ch.

The complexing agents benzotriazole (BT) and tolyltriazole (TT) are widely applied as anticorrosive agents. BT and TT occur in most municipal wastewaters, are only partly removed by wastewater treatment and are, therefore, widely distributed in natural waters [1–3]. BT and TT were determined in wastewaters and in the Glatt River in Switzerland. BT and TT concentrations in primary and secondary effluents of municipal wastewater treatment plants ranged from below 10 to 100 µg/L. Concentrations ranges in grab samples from the Glatt River in ng/L were 640-3'700 for BT and 120–630 for TT. The corresponding mass flows in kg/d were 0.093–1.9 for BT and 0.018–0.36 for TT with clearly increasing trends along the longitudinal river profile. During winter 2003/4, BT loads in weekly composite samples from a station in the lowest stretch of the Glatt clearly indicated the effect of the nearby Zurich airport, where deicing fluids were used, that contained BT as an an anticorrosive additive.

[1] Schaffner C., Giger W., *Chimia* **2004,** 58, 453.
[2] Weiss S., Reemtsma T., *Analyt. Chem*. **2005,** 77, 7415.
[3] Voutsa D., Hartmann P., Schaffner C., Giger W., *Environ. Sci. Poll. Res.* **2006**, 13, in press.

Analytical Chemistry **52**

Nanoscale molecular analysis and chemical imaging at atmospheric pressure: combining SNOM with Mass Spectrometry

Thomas A. Schmitz, Patrick D. Setz, Gerardo Gamez and Renato Zenobi

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

In materials science as well as in the biological sciences, there is great interest and need for spatially resolved chemical analysis and imaging with nanometer resolution. We present a design that combines scanning near-field optical microscopy (SNOM) and mass spectrometry (MS) by utilizing a SNOM tip with an aperture of 50 – 150 nanometers as a light source for laser ablation on the nanometer scale and a transfer ("suction") capillary into the vacuum system. This setup will allow molecular analysis with a spatial resolution given by the size of the SNOM tip aperture.

Our instrument overcomes the limitations of previous SNOM-MS methods for nanoscale analysis: The setup of Kossakovski et al. [1] had to be operated in vacuum and only reached micrometer spatial resolution, whereas our setup allows the analysis to be performed at atmospheric pressure. No other method available today offers this analytical capability, which is crucial for applications in biology, i.e. analysis of metabolites in living cells or tissue. In previous work by our group [2], we demonstrated the feasibility of nanoscale-MS at atmospheric pressure, but analysis at that time was limited in mass spectral performance.

We have now developed a combination of a highly sensitive ion trap and time-of-flight MS for this application. [3] It particularly comprises an optimized inlet and analyte transfer system from atmospheric pressure into the vacuum of the MS. This includes differentially pumped pressure reducing stages and precision positioning of the sampling capillary with respect to the SNOM tip.

We present laser ablation spectra of solid samples, performed with different ionization methods developed for this specific purpose, i.e. conventional electron impact (EI) ionization or chemical ionization (CI). The latter is a particularly soft method and therefore well suited for fragile biological molecules. Our design also enables the utilization of selective ionization methods such as photoionisation in the vacuum far-UV (VUV).

[1] D. A. Kossakovski, et al., *Ultramicroscopy* **1998**, *71*, 111.
[2] R. Stöckle, et al., *Anal. Chem.* **2001**, 73, 1399.
[3] P. D. Setz, T. A. Schmitz, R. Zenobi, *Rev. Sci. Instrum.* **2006**, 77, 024101.

CHIMIA **2006**, *60*, No. 7/8

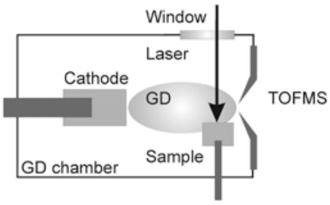
Analytical Chemistry **53**

In situ Laser Ablation Sampling for Pulsed Glow Discharge Time of Flight Mass Spectrometry

Martin Tanner[a], Daniel Fliegel[a], Marc Gonin[b], Katrin Fuhrer[b],
Detlef Günther[a]

[a]Laboratory of Inorganic Chemistry, ETH Zurich, 8093 Zurich, Switzerland
[b]Tofwerk AG, 3602 Thun, Switzerland

Laser ablation (LA) allows sampling of conductive and non conductive targets with high lateral resolution. Pulsed glow discharge (GD) is a dynamic ionization source which provides ionization regimes from electron ionization at the beginning of the GD pulse to penning ionization after extinguished GD pulse. The time of flight mass spectrometer (TOFMS) used in this study provides full mass spectra readout frequencies of less than 1 ms.



Inspired by a study of LA pulsed GD optical emission spectroscopy [1] the effect of GD post ionization on laser generated aerosols from different sample matrices was studied using a TOFMS system. The samples were placed inside the GD chamber between cathode and sampler orifice of the TOFMS (anode). The laser was directed orthogonally to the GD plasma onto the sample. The fast data acquisition allowed studying of the different ionization regimes acting on the LA generated aerosol for single laser shots.

[1] C.L. Lewis, S.K. Doorn, D.M. Wayne, F.L. King, V. Majidi, *Appl. Spectrosc.,* **2000**, *54 (8)*, 1236.

Analytical Chemistry **54**

Mass Spectrometry of RNA: Investigation of Nucleobase- and 2'-Modified Oligo(deoxy)ribonucleotides for Elucidation of the Gas-Phase Dissociation

J. M. Tromp and S. Schürch

Universität Bern, Departement für Chemie und Biochemie,
Freiestrasse 3, 3012 Bern, Schweiz

A wide variety of structural modifications are introduced to antisense oligonucleotides and aptamers to increase their affinity and binding specificity. However, sequence elucidation of chemically modified oligonucleotides cannot be performed by standard protocols. In order to develop mass spectrometry based methods for sequence identification, fundamental mechanistic aspects of gas-phase dissociation of oligonucleotides need to be defined.
Dissociation of oligoribonucleotides (RNA) is characterized by cleavage of the 5'-P-O bond, resulting in the formation of abundant c- and their complementary y-ions [1][2]. The influence of specific structural modifications is investigated using various nucleobase- and 2'-modified oligo(deoxy)ribonucleotides. RNA incorporating a 2'-methoxy-ribose or a 2'-fluoro-ribose show a stabilizing effect of electron-withdrawing 2'-substituents on the N-glycosidic bond, thus, rendering nucleobase loss less prominent. Furthermore experiments demonstrate that the availability of a proton in the vicinity of the phosphate group is mandatory for the RNA typical backbone cleavage. To evaluate the influence of nucleobase loss on gas-phase dissociation, oligo(deoxy)ribonucleotides bearing biphenyl-modifications and abasic sites are studied . Results lead to the conclusion that gas-phase dissociation of RNA is, in contrast to DNA, independent of nucleobase loss. Tandem mass spectrometric experiments support a mechanism where dissociation is initiated by formation of an intramolecular cyclic transition state with the 2'-hydroxyl proton bridged to the 5'-phosphate oxygen [3][4].

[1] R. L. Cerny, K. B. Tomer, M. L. Gross, L. Grotjahn, *Anal. Biochem.*, **1987**, *165,* 175.
[2] S. Schürch, E. Bernal-Méndez and C. J. Leumann, *J. Am. Soc. Mass Spectrom.,* **2002**, *13*, 936.
[3] J. M. Tromp and S. Schürch, *J. Am. Soc. Mass Spectrom.,* **2005**, *16*, 1262.
[4] J. M. Tromp and S. Schürch, *Rapid Commun. Mass Spectrom.,* **2006,** (in press).

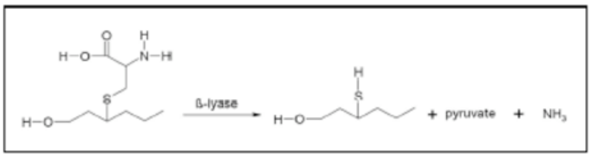
Analytical Chemistry **55**

Determination of the Aroma Precursorby LC/MS in Grape Must of 'Petite Arvine'

Sebastian Völker, Pascal Jacquemettaz, Jean-Luc Lusier, Urban Frey*

University of Applied Science (HEVs), Rte du Rawil 64, CH-1950 Sion

Petite Arvine is an autochthonous grape cultivar used for wines unique to the Canton of Valais in Switzerland. The flavor of Petite Arvine is described as fruity and floral with the major sensory describer to be grapefruit and rhubarb. The major impact aroma compound was identified to be 3-mercaptohexanol at a level of 0.2 to 6 μg/l.[1] However this compound is not present in the must, but is found in the form of a precursor the S-3-(hexan-1-ol)-L-cystein. A published method [2] shows a long sample preparation step and is therefore not adapted for routine analysis.
In this work, we present an isotopic dilution LC/MS method with simplified sample preparation step. The detection limit is 5 μg/l, which is sufficient low compared to the concentration of the precursor found in the must that is in the order of 100 μg/l.
First results and correlation with agronomic parameters are also presented.



Reaction of the aroma precursor to 3-mercaptohexanol. Naturally, only 1 to 10% are converted.

[1] Claudia Fretz, *Ph.D Thesis* ETHZ, **2005**.
[2] Murat, Tominaga, *J. Agric. Food Chem.* **2001**, *49*, 5412.

Analytical Chemistry **56**

Aerosol Transport Efficiency in Femtosecond-Laser-Ablation-Inductively-Coupled-Plasma-Mass-Spectrometry (fs-LA-ICP-MS)

M. Wälle, J. Koch, J. Pisonero, C. Garcia and D. Günther

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

Femtosecond laser ablation ICP-MS has shown its ability to produce accurate analytical data and several studies have been carried out to determine its analytical performance [1-3]. However, until now the transport efficiency of the ablated material to the ICP remained unknown.

Therefore, in this study the transport efficiency in fs-LA-ICP-MS for a Ti:Sapphire laser system operated at its 3rd harmonic (265nm) using a brass sample was determined. To perform these measurements the amount of ablated material was measured by weighing the sample before and after ablation using a micro balance. The produced aerosol was collected using an impactor which contains 13 different stages. The collected aerosol was digested in nitric acid and analyzed by ICP-MS. This approach leads to informations about the transported mass and copper zinc ratio in fractions of different particle sizes, which will be presented and discussed.

[1] R.E. Russo, X.L. Mao, C. Liu, and J. Gonzalez, *J. Anal. At. Spectrom.*, **2004**, *19*, 1084-1089
[2] C. Liu, X.L. Mao, S.S. Mao, X. Zeng, R. Greif, and R.E. Russo, *Anal. Chem.*, **2004**, *76*, 379-383
[3] J. Gonzalez, C. Liu, X. Mao, and R.E. Russo, *J. Anal. At. Spectrom.*, **2004**, *19*, 1165-1168

Analytical Chemistry **57**

ESTROM:
Environmental Science and Technology in Romania

Walter Giger[1*], Denisa Andreea Necula[2], Jacques André Possa[3]

[1]GIGER RESEARCH CONSULTING, 8049 Zurich, Switzerland,
wgiger@hispeed.ch
[2]National Centre of Programme Management, Bucharest, Romania
[3]Swiss National Science Foundation, 3001 Berne, Switzerland

The Swiss National Science Foundation, the Swiss Agency for Development and Cooperation and the Romanian Ministry for Education and Research have launched a scientific collaboration programme called ESTROM, i.e. Environmental Science and Technology in Romania. The programme encompasses nine research projects on inorganic and organic water pollutants focusing on measuring environmental and human exposure and evaluating inputs, fate and behaviour in wastewater, natural waters as well as in drinking water. It concerns dangerous substances as defined in the Water Framework Directive of the European Union, persistent organic pollutants, heavy metals and emerging contaminants such as pharma ceuticals.

Measured environmental and human concentrations shall be the basis for the assessment of risks for the environment and for human health. Thus, the programme will help developing solutions for environmental remediation emphasising chemical pollution of the aquatic environment and will suggest mitigating actions. The outcomes of this programme will help the Romanian authorities to cope with activities of the European Union with respect to the implementation of the Water Framework Directive.

Analytical Chemistry **58**

Endocrine Disrupting Chemicals in Diesel Exhaust

Daniela Wenger[1,2], Andreas Gerecke[1], Norbert Heeb[1], Renato Zenobi[2],
Hanspeter Nägeli[3]

[1]Swiss Federal Institute for Materials Science and Technology (Empa),
Überlandstrasse 129, 8600 Dübendorf, Switzerland
[2]Department of Chemistry and Applied Biosciences, Swiss Federal Institute
of Technology, ETH Hönggerberg, 8093 Zürich, Switzerland
[3]Institute of Veterinary Pharmacology and Toxicology, University of
Zürich, Winterthurerstrasse 260, 8057 Zürich, Switzerland

Endocrine disrupting chemicals (EDCs) are suspected of interfering with the hormone system and therefore causing adverse health effects in humans and animals. Using reporter gene assays, we have investigated the endocrine activity of diesel exhaust. Diesel engines are a source of potential airborne EDCs and inhalable particulate matter, which may be associated with endocrine disruptors. We have analyzed complete exhaust samples from a diesel engine. Samples were collected with and without exhaust gas after-treatment by two particle filter systems.

Our study shows that diesel exhaust contains estrogen receptor ligands as well as aryl hydrocarbon receptor ligands. We measured 1.6 ng 17β-estradiol equivalents and 56 ng 2,3,7,8-TCDD equivalents per m^3 diesel exhaust collected without particulate trap. In diesel exhaust collected with particulate trap, we found 0.8 ng 17β-estradiol equivalents/m^3 diesel exhaust and 9 ng 2,3,7,8-TCDD equivalents/m^3 diesel exhaust. Hence, particle filter systems reduce estrogen receptor ligands in diesel exhaust by around 50% and aryl hydrocarbon receptor ligands by more than 80%.

Analytical Chemistry **59**

Temperature Dependence of Enantiospecific Interactions Occurring During Chiral Separation Studied by ATR-IR Spectroscopy

Ronny Wirz, Davide Ferri, Alfons Baiker

Institute for Chemical and Bioengineering
Department of Chemistry and Applied Biosciences,
ETH Zurich, Hönggerberg, HCI, 8093 Zurich

The rational design of chiral stationary phases (CSP) for high performance liquid chromatography needs a fundamental knowledge of the enantioselective interactions between the CSP and the analyte. A powerful and fast way to investigate the nature of the interaction at the solid/liquid interface crucial for chromatographic separation is attenuated total reflection infrared spectroscopy (ATR-IRS) [1].

We present a study, where ATR-IRS was used in combination with modulation excitation spectroscopy [2] to investigate the interaction of (+/-)-ethyl lactate as the analyte with Chiralpak AS as the CSP in the temperature range 10-50°C. The more strongly bound (+)-ethyl lactate exhibits an enantiospecific interaction with the CSP which is represented by a signal at 1722 cm^{-1}, silent in the case of (-)-ethyl lactate. The intensity of this band decreased at increasing temperature, evidencing a less pronounced interaction. A signal around 1750 cm^{-1} indicated perturbation of the specific functionalized groups of the CSP upon interaction with (+)-ethyl lactate. This signal vanished with increasing temperature.

Fractions of the effluents at the end of the flow through cell have been sampled during one modulation period and analyzed by gas chromatography. In this way, the separation effect could be qualitatively correlated to the spectral information gained at the solid/liquid interface, especially to enantiospecific bonding sites.

[1] R. Wirz, T. Bürgi, and A. Baiker, *Langmuir*, **2002**, *19 (3)*, 785
[2] M. Müller, R. Buchet, U.P. Fringeli, *J. Phys. Chem.*, **1996**, *100*, 10810

Analytical Chemistry **60**

Enhancement of Raman signals with silver-coated tips

Boon-Siang Yeo, Weihua Zhang, Christophe Vannier, Renato Zenobi

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

Since the introduction of tip-enhanced Raman spectroscopy (TERS) in 2000, there has been intense research to push the frontier of this technique towards reproducible nanometer scale chemical analysis. The ability to perform such measurements is of great importance, e.g., for the understanding of catalytic processes and for the fabrication and quality control of molecular electronics. In this work, Ag-coated SiN and SiO_2 atomic force microscope (AFM) tips have been fabricated for use with a TERS setup based on an inverted optical microscope with 488 nm laser excitation. Ag nanoparticles on SiN or SiO_2 rather than Si tips are better for TERS enhancement because of the proximity of the wavelengths of their surface plasmon resonance to 488 nm. Adjustments of the tilt angle of the metallized tip with respect to the surface plane is shown to improve the intensities of the Raman signals. This work serves to enable the more frequent use of the 488 nm laser for chemical analysis with both TERS and fluorescence imaging in the same setup.

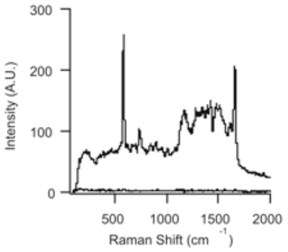


Fig. 1: Raman spectra of brilliant cresyl blue thin film acquired with a Ag-coated SiO_2 AFM tip in contact (dark trace) and retracted (dotted trace). A 60 × increase in Raman signal has been observed.

doi:10.2533/chimia.2006.389 CHIMIA **2006**, *60*, No. 7/8

Analytical Chemistry **61**

Tip-enhanced Raman spectroscopy with Ag tips

Weihua Zhang , Renato Zenobi

Laboratory of Organic Chemistry, EHT Zürich, Wolfgang-Pauli-Strasse 10,
CH-8093 Zürich, Switzerland

We present a systematic study of the performance of Ag tips for tip-enhanced Raman spectroscopy (TERS), which has been attracting the interest of chemists for several years because of its potential to do nano-scale chemical analysis with single molecule sensitivity. In our experiment, a side-illumination scheme was adopted to excite the gap between tip and a metallic sample surface in order to obtain a high field enhancement [1]. Ag tips fabricated by both chemical etching and metallizing sharp glass fiber tips were tested with two wavelengths, 488 nm and 633nm, which are two of the most widely used laser lines. Our experiments show that the etched Ag tips illuminated with the red laser can give a good enhancement for different substrates, such as rough and crystallized, silver and gold surfaces. Clear TERS signals from different monolayers adsorbed on Au or Ag surface can be obtained with 10 sec collection time and low laser power (0.5 mW). With such an enhancement, real-time investigation of chemical reactions on metal surfaces is within reach.

[1] P. K. Aravind and H. Metiu, *Surf. Sci.* **1983**, *124*, 506.

Analytical Chemistry **62**

Railway induced particulate emissions: A one year WD-XRF survey in Zürich, Switzerland

C.N. Zwicky[1], P. Lienemann[1], N. Bukowiecki[2], R. Gehrig[1], M. Hill[1],
A. Ulrich[1], A. Wichser[1]

[1]Empa, Materials Science & Technology, CH-8600 Dübendorf
[2]PSI, Paul Scherrer Institut, CH-5232 Villigen PSI

Public transportation systems are promoted especially in urban areas to reduce the use of individual vehicles. Compared to light duty vehicles, trains operated by electric engines have obviously no emissions of combustion aerosols per passenger and distance. Nevertheless, particulate emissions caused by railway traffic are detectable. Material abrasion from tracks, wheels, brakes and the overhead traction line enhance the concentration of typical railway specific elements in ambient air.

To get representative information about the contribution of railway traffic to the local concentrations of particulate matter with particle sizes below 10 micrometers (PM10), a field study was performed covering a time period of one year. The chemical composition of the aerosol samples was determined with WD-XRF. The measurement campaign involved daily sampling at three measuring sites influenced by railway traffic together with an urban background site without local railway exposition (Zeughaus). The sampling sites were situated at the entry to the main railway station of Zürich (Röntgenstrasse, Gamperstrasse) as well as at a very busy railway line with more than 700 trains per day (Juchhof). The aerosol particles were sampled on quartz filters in a distance of ~10 m from the railway tracks. To study the distance dependence of the railway induced concentrations of railway relevant elements such as iron, manganese and copper, additional samples were taken at Juchhof in a distance of 36 m and 120 m from the railway track. The WD-XRF results coincide well with hourly concentrations measured simultaneously at the same locations by RDI-SR-XRF [1].

[1] N. Bukowiecki et al., *Environ. Sci. Technol.* **2005**, *39*, 5754.

Medicinal Chemistry **63**

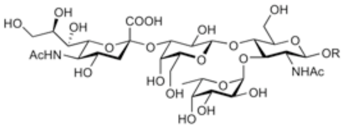
Exploring the Binding Site of E-Selectin

Lionel Tschopp[a], John T. Patton Jr.[b], John L. Magnani[b], Beat Ernst[a]

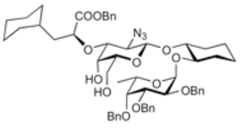
[a]Institute of Molecular Pharmacy, Pharmacenter of the University of Basel,
Klingelbergstrasse 50, CH-4056 Basel, Switzerland
[b]Glycomimetics Inc., Gaithersburg, MD, USA

Selectins play a major role in the adhesion of leukocytes to the vascular endothelium in the early stage of the inflammatory cascade, associated with a number of diseases and pathological situations [1,2]. The sialyl Lewis^x (sLe^x, **1**) epitope, found on the surface of leukocytes is known to be a natural ligand of E-selectin [2].

Here, we present the synthesis of sLe^x mimics with improved pharmacokinetic and pharmacodynamic properties. First, the synthesis of a suitable azide-bearing precursor **2** was developed. The scaffold was then coupled to various alkynes using click chemistry [3] in order to get a small library of triazoles. The inhibitory activity of these compounds was then evaluated in a static cell-free bioassay.



1



2

[1] R. P. McEver, *Glycoconj. J.*, **1997**, *14*, 585.
[2] L. Phillips, E. Nudelman, F. A. Gaeta, M. Perez, A. K. Singhal, S. Hakomori, J. Paulson, *Science*, **1990**, *250*, 1130.
[3] V. V. Rostovtsev, L. G. Green, V. V. Fokin, K. B. Sharpless, *Angew. Chem. Int. Ed. Eng.,* **2002**, *41*, 2596.

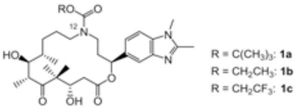
Medicinal Chemistry **64**

Design and Synthesis of 12-Aza-Epothilones (Azathilones)

Fabian Feyen, Jürg Gertsch, Markus Wartmann, Karl-Heinz Altmann

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

Owing to their potent cytotoxicity against tumor cells and a taxol-like mode of action, the epothilones have attracted considerable research interest over the last few years. Several phase I-III clinical trials are ongoing with epothilone B and closely related analogues thereof. In contrast to these closely related structures and based on existing SAR data, our research aims at the creation of new structural scaffolds for microtubule stabilization, which could offer the potential for distinctly different overall pharmacological profiles. These considerations resulted in compounds **1a-1c** as initial targets for total synthesis and biological investigation.



Given the degree of structural divergence from the natural epothilone template, these analogs (which have been termed “azathilones”) may be considered as members of a distinct group of hypermodified natural products with unique structural features. In this presentation we will report on the total synthesis of aza-macrolides **1a-1c**, either based on ring-closing olefin metathesis (RCM) as the key step or *via* a macrolactonization approach. All three compounds prepared proved to be highly potent inhibitors of human tumor growth *in vitro*, with **1a** being at least as potent as epothilone A, both in terms of cytotoxicity and tubulin polymerization. They should thus be attractive new lead structures for anticancer drug discovery.[1]

[1] Feyen, F.; Gertsch, J.; Wartmann, M. Altmann, K.-H. *Angew. Chem.* **2006**, in press.