Invited lecture **5**

Invited lecture **6**

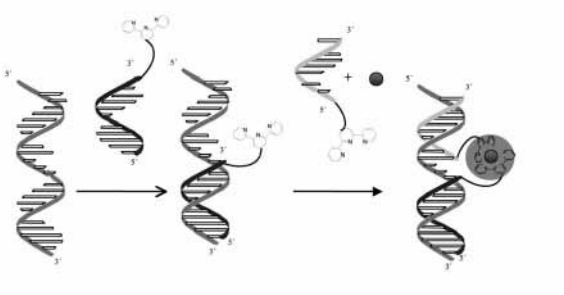
Analytical Chemistry **7**

Oligonuclotide-Arrays: Use of Oligonucleotide-Metal Conjugates for the Detection of DNA

Gapian Bianké[b], Valerie Chaurin[a], E.C.Constable*[a], Maxim Egorov[c], Robert Häner [b], Catherine Housecroft*[a], J. Lebreton[c]

[a]University of Basel, Spitalstrasse 51, 4056 Basel, Switzerland
[b] University of Bern, Freiestrasse 3, 3012 Bern, Switzerland.
[c]Laboratoire de Synthèse Organique, 2, rue de la Houssinière, 44322 Nantes Cedex 03, France

The project is aimed at the preparation and investigation of novel types of arrays containing oligonucleotide metal conjugates. Arrays of this type should allow the *direct* detection and quantification of nucleotic acids (DNA). The basic concept consists of the generation of a fluorescent metal complex upon hybridisation of analyte nucleic acid to substrate bound oligonucleotides containing metal binding ligands in the presence of a second, oligonucleotide-ligand conjugate and appropriate metal ion.



Analytical Chemistry **8**

Metal ions-peptides complexes study by micro-chip ESI-MS

M. Prudent, T. C. Rohner, C. Roussel, H. H. Girault

Laboratoire d'Electrochimie Physique et Analytique, EPFL, 1015 Lausanne, Switzerland

Mass Spectrometry (MS) has taken an important place in analytical chemistry and especially in biomolecules analyses, thanks to the development of soft ionization techniques such as electrospray ionization (ESI). For example, ESI-MS was found to be a suitable technique to study non-covalent complexes as it allows the transfer of the analyte from the solution to the gas phase without significant fragmentation. Metal-peptides interactions, which are involved in many biological functions, were also explored by ESI-MS. Since many years, our laboratory has been being active into the design and the conception of micro-fabricated ESI sources [1]. These original micro-chips coupled to MS were found to be an interesting tool for the identification of several proteins contained in a mixture. In this specific work, the inherent electrochemistry of the electrospray was explored to electrogenerate specific probes which when reacting with peptides coming from the protein mixture digest allows a better protein identification [2]. More recently, theses kind of micro-systems were applied to study the on-line complexation of transition metal with peptides. In that design, a sacrificial metal electrode, which plays the role of metal ions production and electrospray generation, was integrated to the micro-chip. Several transition metal electrodes such as iron, copper, silver were used to determine the specific binding sites of the corresponding ions with peptides [3].

[1] T. C. Rohner, J. S. Rossier and 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] T. C. Rohner and H. H. Girault, *Rapid Commun. Mass Spectrom.* **2005**, *19*, 1183.

Analytical Chemistry **9**

Comparison of various powder preparation techniques for LA-ICP-MS analysis

Christian Frei, Detlef Günther

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

High purity materials became more and more important in the last decades driven by higher quality requirements for example in coating industry.
LA-ICP-MS is a promising analysis technique for the analysis of high purity solid materials with detections limits in the low ppm range for most of the elements [1]. LA-ICP-MS has the advantage that no extensive sample digestion is necessary, which saves time and reduces the risk of contamination.
To control the industrial production process of coating materials like TiO_2 with a “claimed” high purity, it is important to have the possibility to analyse all intermediate products including powders with an accurate and fast analytical method.

The analysis of powder samples using LA-ICP-MS is still an difficult task, due to the less-controlled ablation process. In this work, different possibilities to convert a powder into a solid ablation target were compared. The results of direct pressed TiO_2 powder samples were compared with $Li_2B_4O_7$ fluxed TiO_2 powder samples and pressed mixtures of TiO_2 with polyethylen, used as a binder, to improve the sample stability.
Ablation experiments were carried out using an 193nm ArF Excimer lasersystem in combination with an Elan 6100 ICP-MS.
The results are discussed with respect to signal stability, sample homogeneity, accuracy and detection limits.

[1] Becker JS, Dietze HJ, *INTERNATIONAL JOURNAL OF MASS SPECTROMETRY* , **2003**, 228 (2-3): 127-150

Analytical Chemistry **10**

Measurement of Intact, High Mass Protein Complexes by MALDI Mass Spectrometry

Ryan J. Wenzel, Alexis Nazabal, Renato Zenobi

ETHZ, Wolfgang-Pauli-Strass 10, 8093 Zürich, Switzerland

Normally to analyze protein complexes in their native state it is necessary to work with special 'soft' MALDI conditions. Additionally, to analyze samples which produce ions over a broad range at high m/z the sensitivity is often not adequate using standard MALDI instrumentation. A recently developed MALDI time of flight mass spectrometer was employed for sensitive high mass analysis using cryogenic superconducting tunnel junctions (STJ) (macromizer™, Comet AG). We developed a highly efficient chemical cross-linking protocol that, in effect, "locks" the sample in its native state for analysis. This prevents the protein complexes from dissociation during MALDI ionization, which is usually the case.

Various protein complexes were examined ranging from pure single component samples to human blood serum. The issue of specific binding when using this stabilizer is addressed in detail with analysis of all samples showing dominantly specific binding. Several samples of monoclonal antibodies (150kDa) specifically bound to their corresponding antigens will be presented, demonstrating various sized antigen such as insulin (6kDa), prion protein from a mouse (23kDa), bovine serum albumin (66kDa) and human serum albumin (66kDa). Each sample shows only binding of the correct antigen to its mate, whether done in pure solution or in complex mixtures such as human blood serum. Analyses of stabilized complexes consisting of multiple secondary antibodies bound specifically to another antibody-antigen complex are also demonstrated, showing signals extending above 450kDa. Complicated protein structures were designed using lumazine synthase (17kDa) to create multimeric protein constructs in excess of 1MDa. These samples show specific signal’s due to the desired complexes and demonstrate the ease of use and benefit of the chemical stabilization process for complexes of different sizes and complexity.

Analytical Chemistry **11**

Rapid determination of pK_a values by capillary zone electrophoresis

Yveline Henchoz [a,b], Laurent Geiser [a], Pierre-Alain Carrupt [b], Jean-Luc Veuthey [a]

[a] Laboratoire de Chimie Analytique Pharmaceutique, Ecole de Pharmacie Genève-Lausanne, Section des sciences pharmaceutiques, Université de Genève, Bd d’Yvoy 20, CH-1211 Genève 4, Suisse, http://www.unige.ch/sciences/pharm/fanal.
[b] LCT-Pharmacochimie, Ecole de Pharmacie Genève-Lausanne, Section des sciences pharmaceutiques, Université de Genève, Quai Ernest-Ansermet 30, CH-1211 Genève 4, Suisse, http://www.unige.ch/pharmacochimie.

Physico-chemical properties of pharmaceutical compounds are needed to predict ADME (absorption, distribution, metabolism and excretion) behaviour; among these properties, ionization constants (pK_a) are essential to understand pH-related permeation mechanisms. Capillary zone electrophoresis (CZE) permits the measurement of pK_a values in a simple automated way. Moreover, this method does not require a good sample purity and solubility, and needs only a low amount of solvent and sample. A plot of the effective mobility measured versus pH of the background electrolyte (BGE) enables the determination of pK_a values.

Due to the tremendous increase of new chemical entities (NCE’s), there is a particular demand for rapid physico-chemical profiling. A strategy was investigated to reduce the time needed for pK_a determinations by CZE. For this purpose, short-end injection on a dynamic coated capillary was used. Different BGEs were tested, from 1.4 to 11.3 and from 10 to 100 mM for pH range and ionic strength, respectively. Under these conditions, determined pK_a values were in good agreement with the literature values. Therefore, short-end injection and dynamic coating procedure are of great interest for routine pK_a value determination on a commercially available instrument.

Analytical Chemistry **12**

Desorption electrospray ionization mass spectrometry (DESI-MS) : direct drug tablets analysis

Luc Alexis Leuthold, Jean-François Mandscheff, Emmanuel Varesio and Gérard Hopfgartner

Life Sciences Mass Spectrometry, School of Pharmaceutical Sciences, University of Geneva, 20 Bd d’Yvoy, 1211 Geneva 4, Switzerland

Desorption electrospray ionization (DESI), a recently developed technique [1], was tested for the rapid confirmatory analysis of drug tablets, without sample preparation or separation.
DESI-MS experiments were performed with an home-made DESI source coupled to a quadrupole linear-ion trap instrument (Q TRAP, AB / MDS Sciex). Ionization and desorption parameters were optimized with test compounds. Twenty commercial drugs were tested so far using methanol/water or acetonitrile/water sprays with Information Dependant Acquisition (IDA). Spectra showed usually almost exclusively the pseudo-molecular ion of the drug after directing a pneumatically-assisted electrospray onto the tablets surface, under ambient conditions. Directly triggered MS/MS spectra were used for confirmation, with an analysis time that could go under 10 seconds. If needed, MS^3 experiments were carried out while the tablet stayed under the spray. Detection limits were sub-nanogram. Ecstasy and an analog were identified in illicit tablets.
These results show that DESI can be easily used to analyze drug tablets and powders. The method is very fast, highly selective and requires no sample preparation. With MS/MS spectra library comparison, this technique could become very powerful for the almost instant analysis of drug or unknown tablets.

[1] Zoltán Takáts, Justin M. Wiseman, Bogdan Gologan and R. Graham Cooks, *Science* **2004**, *306*, 471.

Analytical Chemistry **13**

Study of Conformations of Biomolecules in the Gas Phase by Fluorescence Resonance Energy Transfer

Maxim Dashtiev, Vladimir Azov, Vladimir Frankevich, Ludwig Scharfenberg and Renato Zenobi*

Department of Chemistry and Applied Biosciences, Swiss Federal Institute of Technology, ETH Hönggerberg, CH-8093 Zürich, Switzerland

Study of conformations of biomolecules by fluorescence resonance energy transfer (FRET) [1,2] in the gas-phase has attracted great attention since it allows to compare gas-phase and solution phase structures, as well as elucidate the effect of the solvent on the molecule. Combining FRET with mass spectrometry is very challenging but it has a number of advantages, such as the capability of (a) isolation of ions and elimination of unwanted species prior detection and (b) identification of the molecule by its mass. For successfully observing FRET in the gas-phase it is important to find suitable fluorophores. In this study, several rhodamine dyes were examined, and the correlation between solution phase and gas-phase fluorescence data was established. This is the first time that FRET in gas-phase ions is demonstrated unambiguously.

[1] L. Stryer, *Ann. Rev. Biochem.* **1978**, *47*, 819-846.
[2] Valeur, B. *Molecular Fluorescence*; Wiley:VCH Verlag GmbH, **2001**; p. 247

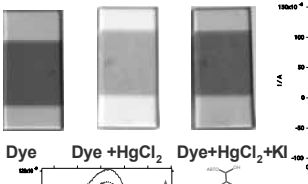
Analytical Chemistry **14**

Highly Selective and Reversible Optical, Colorimetric and Electrochemical Detection of Mercury (II) by Amphiphilic Ruthenium complexes Anchored onto Mesoporous Oxide Films

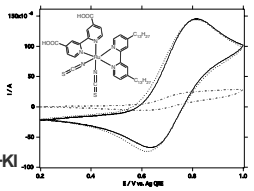
Md. K. Nazeeruddin, D. Di Censo, R. Humphry-Baker, and M. Grätzel

Laboratory for Photonics and Interfaces, Institute of Chemical Science and Engineering, Ecole Polytechnique Fédérale de Lausanne, CH-1015 Lausanne, Switzerland

The fluorimetric, colorimetric and electrochemical detection of mercury ions with functionalized ruthenium sensitizers in aqueous, and nonaqueous solutions, and anchored TiO_2 films were investigated. It was found that mercury ions coordinate reversibly to the ruthenium sensitizers, which induces a color change and increases phosphorescence intensity significantly. The detection limit for mercury(II) ions using emission and UV-visible spectroscopy in homogeneous aqueous solutions is estimated to be ~20 ppb. The results shown herein have important implications in the development of novel reversible sensors based on nanocrystalline semiconductor films for simple, swift and selective detection and decontamination of mercury ions in aqueous solutions.



Emission spectral changes of the N719 dye as a function of increasing amounts of Hg^{2+} ions.



Cyclic voltammogram of the N621 complex (black line), exposed to the solutions of $HgCl_2$ (red dashed dotted line) and $Pb(ClO_4)_2$ (blue dashed line).

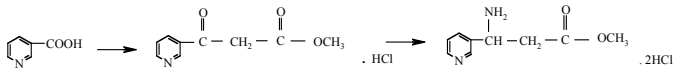
Analytical Chemistry **15**

Metal Analyses in Environmental and Pharmaceutical Samples by Capillary Electrophoresis

Gamze Kavran Belin, Fazil Osman Gülaçar

Laboratory of Mass Spectrometry, University of Geneva, Sciences I, Boulevard d'Yvoy 16, CH-1211 Geneva 4, Switzerland.

Determination of metal ions is of great interest in environmental, medical and pharmaceutical sample analyses. Capillary electrophoresis (CE) has a number of advantages like greater separation efficiency, higher selectivity, simplicity and adaptability to a variety of different application conditions. Difficulty of the analyses of metal ions by CE is that most of them have roughly same mobility because of their similar size and charge density. In order to modify the mobilities of alkali and alkaline-earth metal ions, either complexation or ion-pairing reagents [1,2] are widely used. In this work, we investigated a new CE approach by synthesizing and using a new ion-pairing reagent, methyl 3-amino-3-(3-pyridyl) propanoate dihydrochloride (MAPP), in the running electrolyte for the separation and determination of some common metal ions and the method was applied to the analyses of pharmaceutical vitamins and various water samples.



Nicotinic acid Methyl nicotinoacetate HCl MAPP

[1] T. Okada, *J. Chromatogr. A* **1999**, *834*, 73.
[2] T. Soga, G. A. Ross, *J. Chromatogr. A* **1999**, *834*, 65.

Analytical Chemistry **16**

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

Cédric Bovet, Arno Wortmann and Renato Zenobi

Department of Chemistry and Applied Biosciences, Swiss Federal Institute of Technology (ETH), 8093 Zürich

Estrogens are a group of steroid hormones that affect growth, differentiation and the development of reproductive tissues by binding to the estrogen receptor (ER). A variety of chemicals released into the environment, known as endocrine disruptors, can mimic the action of steroid hormones by binding to the ER. Due to the negative effects of these endocrine disruptors on wildlife and humans, we are developing a screening method based on electrospray ionization mass spectrometry (ESI-MS) to evaluate quantitatively the affinity of hundreds of possible disruptors with ER.

In its native state, ER forms a homodimer that noncovalently binds the ligand in the ligand binding domain. The crystal structure of a triple mutant human estrogen receptor ligand binding domain (hER LBD) shows a similar structure and binding affinity than the wild type form [1]. We have successfully measured with matrix assisted laser desorption ionization mass spectrometry (MALDI-MS) the monomer ($m/z = 44$ kDa) and the homodimer ($m/z = 88$ kDa) of this mutant hER LBD. We are also establishing the technical basis for the detection of noncovalent complexes of the hER LBD with electrospray ionization mass spectrometry (ESI-MS). This soft ionization technic allows, under suitable conditions, the detection of noncovalent complexes in the gas phase and an estimation of the binding strength.

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

Analytical Chemistry **17**

Multi-tagging of cysteine units within a microspray emmiter

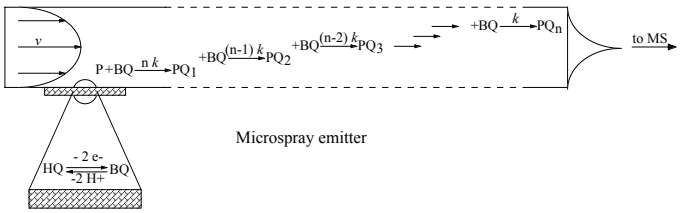
L. Dayon, C. Roussel, J. Josserand and H.H. Girault*

Laboratoire d'Electrochimie Physique et Analytique, EPFL, CH-1015 Lausanne, Switzerland

Benzoquinone probes (BQ) electrogenerated from hydroquinones (HQ) at a microspray electrode react with the thiol functions of biomolecule (P) and the products (PQ_i) are analysed *via* mass spectrometry [1, 2, 3].

Experiments with unmodified β-lactoglobulin A and its chemically reduced form highlight the strong effect of the cysteine site reactivity on the tagging efficiencies. Chemically reducing the protein prior to tagging leads both to an increase in the reaction kinetics and to a consecutive tagging of the cysteine units liberated by the reduction of disulfide bonds.

A finite-element model is used to simulate the distribution of the tagged species concentration in the microchannel before the spray event. The parameters determining the kinetics of the tagging are assessed and discussed considering the microfluidic aspects of the process.



[1] L.Dayon, C. Roussel, H.H. Girault, *Chimia* **2004**, *58*, 204.
[2] C. Roussel, L. Dayon, N. Lion, T.C. Rohner, J. Josserand, J.S. Rossier, H. Jensen, H.H. Girault, *J. Am. Soc. Mass. Spectrom.* **2004**, *15*, 1767.
[3] L. Dayon, C. Roussel, M. Prudent, N. Lion, H.H. Girault, *Electrophoresis* **2005**, *26*, 238.

Analytical Chemistry **18**

Direct Analysis of immunocomplexes by MALDI ToF MS

Alexis Nazabal, Ryan Wenzel and Renato Zenobi
Department of Chemistry and Applied Biosciences, ETH, Zürich, Switzerland.

The study of intact protein complex by mass spectrometry stills challenging because of the tendency of the complexes to dissociate during the ionization steps. We present a new method combining cross-linking chemistry to stabilize protein complexes before ionization and MALDI mass spectrometry equipped with a superconducting tunnel junction detector. As a first model to evaluate this new method, we have analyzed Antibodies/Antigen complexes, performing sandwich assays, competition assays for epitope mapping and binding kinetics. Only 10 minutes after starting the binding reaction between the antibodies and the antigens, we have detected the specific complexes (AntiHSA/HAS, AntiBSA/BSA, 6H4/bPrP). The mass spectra of the reaction 6H4/bPrP is constituted of two major peaks in the range 170-210Kda representing the monoclonal antibody interacting specifically with one or two prion proteins. For sandwich assay the complex 6H4-2bPrP-3B8 have been detected with a strong peak at m/z=357 kDa. Our method has also been tested successfully for epitope mapping by competition assays. For binding kinetics, it is possible to follow on the same spectra the evolution of the complexes 6H4-bPrP and 6H4-2bPrP as a function of incubation time.

Analytical Chemistry **19**

Nanoscale Chemical Imaging with Tip-Enhanced Raman Spectroscopy

Boon Siang Yeo, Christophe Vannier, Renato Zenobi*

Swiss Federal Institute of Technology Zürich, Department of Chemistry and Applied Biosciences, CH-8093 Zürich, Switzerland

The ability to perform nanoscale chemical analysis is of great importance towards the understanding of catalytic processes and for the fabrication and quality control of molecular electronics [1]. In this work, atomic force microscopy (AFM) and Raman spectroscopy has been coupled to demonstrate the identification of chemical functionalities of adsorbates with nanometer spatial resolution. The vital component in this technique is the Raman scattering enhancing AFM probe, fabricated in various ways, e.g. by attaching a silver nanoparticle of the optimum size on the tip apex. An application of this technique is shown here for surface-bound nanostructures.

[1] R. Zenobi and V. Deckert, *Angew. Chem. Int. Ed.* **2000**, *39*, 1746.

Analytical Chemistry **20**

Atmospheric pressure desorption/ionization on silicon mass spectrometry in metabolomics on a single cell level

Andrea Amantonico and Renato Zenobi

Department of Chemistry and Applied Biosciences, Swiss Federal Institute of Technology (ETH), 8093 Zurich

Despite a large body of knowledge about cellular components little is known about how all these work together as a system. To obtain a better understanding of system biology is necessary to integrate experimental data into predictive models. Almost all current methods of quantification aggregate data from an entire cell population, while even cells in monoclonal cultures display strong differences on all levels. Thus a single cell approach to '-omics' research is essential to create accurate models.

We intend to develop mass spectrometry (MS) based instrumentation for metabolomics on a single cell level. To achieve this aim we propose a direct coupling of a microfluidic chip, for cell processing and sample work-up, to an innovative chip/MS interface based on a recently developed matrix free laser desorption/ionization technique from the surface of porous silicon (DIOS) [1]. DIOS-MS can potentially reach the high sensitivity required for detection and perhaps even quantification of metabolites in a single cell and can be easily interfaced with microfluidic devices fabricated of silicon for a direct detection of the analytes. Moreover, as DIOS-MS does not require a matrix to assist the desorption and ionization, mass spectra with a low chemical background at low mass range can be produced and therefore reliable analysis of low molecular weight compound, such as metabolites, is possible.

[1] J. Wei, J. M. Buriak, G. Siuzdak, *Nature* **1999**, *399*, 243-6.

Analytical Chemistry **21**

Comparison of MOS-based Electronic Nose vs. SPME-GC-MS Measurements of Commercial and Self Prepared Citrus Juices

Hans Reinhard, Fritz Sager, Otmar Zoller

Swiss Federal Office of Public Health, Division of Food Science, 3003 Bern, Switzerland

Fruit juices and beverages, but especially citrus juices were classified by discriminant analysis using the volatile juice constituents determined by SPME-GC-MS and an electronic sensor instrument. The results from these two techniques were compared. Generally, the electronic sensor instrument turned out to be less successful in classification under the experimental conditions chosen. On the other hand different separation patterns evolved from the two techniques, what lets them appear complimentary. Sample throughput and data analysis was favored by the automated electronic sensor instrument, while for SPME-GC-MS only partially automated sample measurement and data handling was available what rejects its applicability for routine purposes.

Recently, we reported the feasibility of differentiating composition, origin and harvest of citrus fruits and their juices by SPME-GC-MS and chemometrics [1]. In this context an electronic nose instrument was tested and data compared to those gained with the SPME-GC-MS technique. This data comparison will be presented.

[1] H. Reinhard, F. Sager, O. Zoller, *Mitt. Lebensm. Hyg*. **2004**, *95*, 632.

Analytical Chemistry **22**

FTIR for High Precision Trace Gas Analysis

Joachim Mohn and Lukas Emmenegger

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

Fourier Transform Infrared (FTIR) spectroscopy is a versatile tool for gas analysis. Applications are widespread from real-time measurement of industrial processes to environmental monitoring of trace gases [1, 2]. The quality of the analytical result, however, is strongly dependent on task-specific optimization of the system which includes hardware configuration, quantification algorithm and calibration strategies.
We developed a method based on a portable FTIR spectrometer which allows the simultaneous on-site analysis of multiple trace gases (CO_2, CH_4, CO, N_2O) as well as the $^{13}C/^{12}C$ isotopic ratio of atmospheric CO_2. ($\delta^{13}CO_2$). The hardware configuration includes a carefully selected gas cell, optical filters and nitrogen-cooled detectors (MCT-A, InSb). Different quantification algorithms based on classical least square (CLS) and partial least square (PLS) techniques were compared. Special attention was paid to the preparation of suitable reference gases for $^{12}CO_2$ and $^{13}CO_2$ starting with CO_2 from sources with characteristic isotopic composition, such as the combustion of petrol, wood or natural gas.
Applicability of our approach for ambient air monitoring was demonstrated during an inter comparison study with independent measurement techniques including GC-FID/ECD and a pulsed quantum cascade laser. Special attention was given to the precise determination of $\delta^{13}CO_2$ because of its potential to study processes with distinctive isotopic signatures.

[1] J. Mohn, U. Beck, K. Zeyer, L. Emmenegger, J. Mol. Structure, **2005**, *744-747*, 247-253.
[2] J. Mohn, A.-M. Forss, S. Brühlmann, K. Zeyer, R. Lüscher, L. Emmenegger, M. Weilenmann and N.V. Heeb, Int. J. of Environment and Pollution, **2004**, *22*, 342-356.

Analytical Chemistry **23**

Are Polymers the Major Components of Atmospheric Organic Aerosols ?

A. Reinhardt, M. Kalberer, R. Zenobi

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

The analysis of trace organic compounds in the environment is an area of increasing importance. Of particular interest to us is the analysis of organics in the atmosphere and especially in organic aerosol particles. Organic aerosols are of importance in such different fields as global climate change, regional air quality and human health. A large fraction of the organic aerosol is emitted directly in particulate form by combustion processes. However, significant amounts are also formed in the atmosphere as a result of oxidation reactions of volatile organic compounds resulting in low volatility compounds. Despite their importance there is currently little knowledge about the composition of organic aerosols. To study the complex compounds mixtures present in organic aerosols, we perform controlled laboratory experiments in a smog chamber, built in collaboration with the Laboratory of Atmospheric Chemistry at the Paul Scherrer Institute. Regular mass patterns with distinct differences of 14 or 16 mass units were observed by laser ionization mass spectrometry for oligomers formed from precursors like α-pinene and isoprene. Those oligomers were found to have molecular masses of up to about 600 Da, which is similar to oligomers formed from aromatic precursors [1]. We currently focus our work on the characterization of these polymers an online of HPLC and an ESI-Q-TOF instrument.

[1] Kalberer M., Paulsen D., Sax M., Dommen J., Fisseha R. Prevot A. S. H., Frankerich V., Zenobi R., and Baltensperger U., (2004), **2004,** 303 159-1662.

Analytical Chemistry **24**

Quantum-Cascade Lasers for Field Measurements of N_2O and CO_2

Lukas Emmenegger and Kerstin Zeyer

Empa, Swiss Federal Laboratories for Materials Science and Technology, CH-8600 Dübendorf, Switzerland

Quantum-cascade lasers (QCLs) are novel light sources that open promising options based on the fundamental ro-vibrational absorption bands of most gases in the mid-infrared range. Our work is focused on laboratory and field tests using a QCL at 4.46 μm and a HgCdZn detector, which are both thermoelectrically cooled and thus suited for extended field operation [1].
The QCL was driven with short (~ 10 ns) pulses and a 1% duty cycle at a temperature of approximately -25 °C. Extractive samples were measured at 65 mbar in a 0.5 l astigmatic multipass absorption cell with a path length of 56 m. Spectral scans were obtained by a sub-threshold voltage ramp which creates a bias temperature. The relevant parameters were chosen to allow simultaneous detection of N_2O and CO_2 at ambient concentrations.
Laboratory tests showed excellent linearity and no detectable cross-sensitivity to CO_2 or H_2O. Quantification based on HITRAN [2] parameters differed by up to 20 % when compared to certified calibration gases. After stabilization of the optical bench and optimization of the electronics we obtained a minimum Allan variance corresponding to 0.12 ppb (0.05 %) or 0.56 ppm (0.19 %) for N_2O and CO_2, respectively. Field validation for ambient air was done in comparison with FTIR and GC-FID/ECD. Fast measurements of up to 20 Hz, high precision and selectivity make this an exciting analytical tool for the continuous measurement of many substances, including the isotope ratio $\Delta^{13}C/^{12}C\text{-}CO_2$.

[1] Nelson, D.D., McManus, B., Urbanski, S. et al., *Spectrochimica Acta A* **2004**, 60, 3325-3335.
[2] Rothman, L.S., Barbe, A., Benneret al., *Journal of Quantitative Spectroscopy & Radiative Transfer* **2003**, 82, 5-44.

Analytical Chemistry **25**

Tip-Enhanced Raman Spectroscopy in the 'Gap-Mode'

Weihua Zhang, Christophe Vannier, Renato Zenobi*

Department of Chemical and Applied Biosciences, Swiss Federal Institute of Technology, ETH Hönggerberg, 8093 Zürich, Switzerland

High spatial resolution Raman spectroscopy is a promising technique for analyzing the chemical properties of nano-materials such as biopolymers, carbon nanotubes, etc. In this work, we present 'gap-mode' tip-enhanced Raman Spectroscopy (TERS), in which the gap between a metalized tip and a metal substrate is illuminated from the side to excite Raman Scattering. The gap size which is tunable in range of 0-10nm is controlled by shear-force using a tuning fork. With optimum gap-width, tip shape and polarization of the illumination, the gap-mode is excited, which induces a $10\text{-}10^2$ times enhancement of electromagnetic field in the gap. Consequently, the enhancement of Raman signal is larger than 10^4 times. Comparing to traditional bottom illumination setup of TERS[1,2,3], 'gap-mode' TERS gives higher Raman enhancement and better spatial resolution due to the higher EM field enhancement and better field confinement by the gap than by an isolated metalized tip[4]. Moreover, this technique is also suitable for investigating biosamples as their usually large fluorescence background will be quenched by the metal surface. Using such setup, Raman microscopy with a spatial resolution better than 30nm is demonstrated.

[1] Stöckle, R. M., Suh, Y. D., Deckert, V. and Zenobi, R., *Chem. Phys. Lett.* **2000**, *318*, 131.
[2] Hayazawa, N., Inouye, Y., Sekkat, Z., and Kawata, S., *Opt. Comm.* **2000**, *183*, 333.
[3] Novotny, L., Sánchez, E. J. and Xie, X. S. *Ultramicroscopy*, **1998**, *71*, 21.
[4] Aravind, P. K. and Metiu, H., *Surf. Sci.* **1983**, *124*, 506.

Analytical Chemistry **26**

Investigation of MALDI sample preparations for the analysis of noncovalent protein complexes

Arno Wortmann, Tatiana Pimenova, Christophe Vannier, Renato Zenobi

ETH Zürich, ETH Hönggerberg, HCI-D330, CH-8093 Zürich, Switzerland

Different sample preparation procedures have been investigated for the use in matrix assisted laser desorption ionization mass spectrometry (MALDI-MS) analysises of non-covalent protein complexes. So far MALDI-MS has only found limited use for the analysis of protein complexes [1], because of the difficulties in maintaining noncovalently bound complexes intact during sample preparation, subsequent ionization and mass analysis. Here we use confocal laser scanning microscopy (CLSM) to investigate the distribution of analytes within the MALDI crystal samples [2]. By using fluorescent resonance energy transfer we are also able to distinguish between intact and dissociated protein complexes within these crystals. This approach is used to optimize existing sample preparation and analysis-protocols [3]. Moreover, intact complexes are often observed only for the first laser shot on a given area and specific morphology in a MALDI crystal. We are currently investigating the generality of this phenomenon with MALDI-MS as well as CLSM.

figure: CLSM image of a ferulic acid crystal with an equimolar mixture of streptavidin conjugates with two fluorescent labels. The emission in the red channel (right) shows only the intact tetrameric complexes, while monomers are visible in the green channel (left). The image shows that in this exampe the noncovalent complex survives the crystallisation process.

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[2] Y. Dai et al., *Anal. Chem.,* **1996**, *68*
[3] M. Zehl et al., *Anal. Chem.*, **2005**, *77*

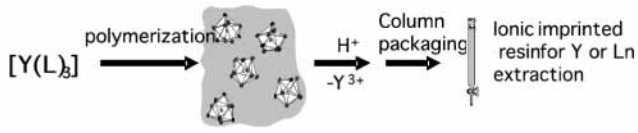
Analytical Chemistry **27**

Selective extraction of yttrium-90 and lanthanide cations with an ion-imprinted polystyrene resin.

Anne-Sophie Chauvin,[a] Jean-Claude G. Bünzli,[a] Jean-François Valley,[b] Rosario Scopelliti,[a] Pascal Froidevaux[b*]

[a] Laboratory of Lanthanide Supramolecular Chemistry, Ecole Polytechnique Fédérale de Lausanne, BCH 1405, 1015 Lausanne, [b]Institute of Applied Radiophysics, University of Lausanne, Grand Pré 1, 1007 Lausanne,

Extraction and separation of metal cations have always been challenging problems, in particular the highly selective separation of yttrium-90 from its parent isotope, strontium-90 for the design of radiopharmaceuticals, or of lanthanides. Specific needs for analytical procedures for radio-elements led us to the development of styrene-based ion-imprinted resins featuring coordination cages which are specifically tailored for hosting trivalent yttrium and built from three helically wrapped vinyl derivatives of dipicolinic acid. High-resolution Eu-luminescence experiments revealed that the preformed geometry of the complexation sites is well preserved in the imprinted polymers. The ion- imprinted polymer proved to be particularly well adapted for yttrium extraction (8.9 ± 0.2 mg/g resin), with a fast rate of extraction ($t_{1/2}$= 1.7 minutes). Within the lanthanide series, the resin is also selective towards heavy lanthanides. Finally, it displays a large selectivity for yttrium and lanthanide cations against alkaline and alkaline-earth elements. This selectivity opens startling perspectives for the production of highly pure ^{90}Y and radio-lanthanides for medical applications, and for trace analysis of these radio-chemicals in food and in the environment.



Analytical Chemistry **28**

Evaporative light scattering: A novel detection method for chromatographic analysis of humic like substances in aerosols

Christian Emmenegger, Renato Zenobi, Markus Kalberer

Department of Chemistry and Applied Biosciences, Swiss Federal Institute of Technology, 8093 Zurich, Switzerland

The chemical nature of a large fraction of the mass of organic aerosols is not known. It has been shown that high molecular weight compounds make up a significant fraction (20-50%) of the water-soluble organic carbon (WSOC) fraction of the aerosols. Havers et al. [1] suggested the term humic like substances (HULIS), as their chemical characteristics are similar to humic acids, which are found in natural waters and soils.

HULIS have been analyzed and characterized using various methods. A quantification still remains difficult because their chemical structure is ill-defined. Standard detection methods for liquid chromatography such as UV, require identical or at least structurally similar standards in order to obtain reliable quantitative results, because optical or ionization properties can vary by orders of magnitudes between compound classes. However, for HULIS there are no true standards available. We present a new technique to quantify the HULIS fraction from atmospheric aerosol particles: Size exclusion chromatography (SEC) coupled with Evaporative Light Scattering Detection (ELSD). The ELSD operation principle [2] has three steps: (1) nebulization with a nitrogen gas flow of the column effluent, (2) mobile-phase evaporation in a heated drift tube, leading to a particulate form of the analyte, and (3) light scattering detection. Using standards such as PMA and PEG of different molecular weights, several dicarboxylic acids and levoglucosan as well as a humic acid standard showed that they all fall on one single calibration curve correlating the signal intensity and the concentration. This should allow to determine the HULIS concentration in ambient air.

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[2] Kohler et al. *Trac-Trends Anal. Chem.* **1997**, *16*, 475.

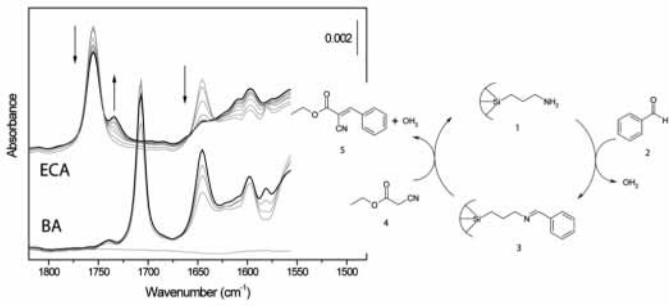
Analytical Chemistry **29**

Monitoring of catalytic solid-liquid interface during heterogeneous Knoevenagel condensation using ATR-infrared spectroscopy

Ronny Wirz, Davide Ferri, Alfons Baiker

Swiss Federal Institute of Technology, Institute of Chemical and Bioengineering, ETH Hönggerberg, HCI, CH – 8093 Zurich, Switzerland

Attenuated total reflection infrared spectroscopy (ATR–IRS) offers the possibility to follow surface processes at solid-liquid interfaces under reaction conditions. The behavior of both continuous and batch reactions can be studied in one single cell of the size of a microreactor.
Here we have used ATR–IRS to investigate the condensation between benzaldehyde (BA, **2**) and ethylcyanoacetate (ECA, **4**) to ethyl-α-cyanocinnamate (ECC, **5**) over γ-aminopropyl-modified silica (AP-SiO_2, **1**). Benzaldimine (**3**, ν(C=N) at 1645cm^{-1}) could be identified as a reaction intermediate. It is formed rapidly between AP-SiO_2 and BA and reacts continuously with ECA to give ECC (ν(C=O) at 1732cm^{-1}).



Analytical Chemistry **30**

Determination of Aldehydic Lipoperoxidation Products: n-alkanals, n-alkenals and 4-hydroxynonenal in Biological Matrices

Sauvain Jean-Jacques, Trinh Vu Duc

Institut Universitaire Romand de Santé au Travail, Bugnon 19, CH - 1005 Lausanne, Switzerland

Oxidative stress is an important process associated with many pathological disorders like inflammation, cancer and aging [1]. This oxidative stress corresponds to the imbalance in the cellular oxidant status, due to the appearance of free reactive oxygen species (ROS), like OH•, H_2O_2. One way these ROS are acting is through the oxidation of the cell lipidic membrane, leading to a wide variety of degradation products, including carbonyl compounds like n-alkanals, n-alkenals and 4-hydroxyalkenals.
At IST, we are interested in assessing the oxidative stress induced by diesel particulates in exposed workers [2]. For that reason, we developed an analytical method enabling the determination of n-alkanals (hexanal, heptanal, octanal, nonanal, decanal), n-alkenals (*trans*-2-nonenal, *trans*-4-decenal) and 4-hydroxynonenal in urine and serum.
Sample preparation is consisting of a derivatization step with pentafluorobenzylhydroxylamine followed by an isolation on reverse phase C18 SPE cartridge. For 4-hydroxynonenal, a further silylation of the hydroxy moiety is achieved. Quantitation is done by GC-MS ion trap.
Except for decanal in serum, good recovery (> 80%) have been achieved for both matrices. With limit of detections in the range of 0.01-0.3 nmol/ml, this method allows the determination of these compounds in physiological and pathological conditions. For example, statistically different ($p<0.05$) levels of hexanal in urine of smokers and non smokers are measured.

[1] B. Halliwell, J.M.C. Gutteridge, *Free Radicals in Biology and Medicine*, Clarendon Press, Oxford, **1985**
[2] J.-J. Sauvain, T. Vu Duc, M. Guillemin, *Int. Arch. Occup. Environ. Health*, **2003**, *76*, 443-455

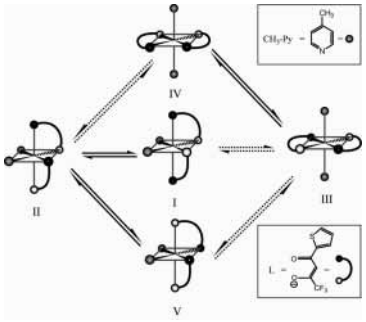
Analytical Chemistry **31**

Isomerisation Mechanisms of a Labile Co(II) Octahedral Complex

Fabrice Riblet[a], Ghenadie Novitchi[a,b], Lothar Helm[a] and André E.Merbach[a]

[a] Laboratoire de Chimie Inorganique et Bioinorganique, Ecole Polytechnique Fédérale de Lausanne, EPFL-BCH, CH-1015 Lausanne, Switzerland [b] Moldo State University, MD-2009, Chisinau (Moldova)

The paramagnetic {*trans*(N)-*trans*(CF_3)-*trans*}-**[CoL_2(CH_3-Py)$_2$]** complex where **HL** is an asymmetric acetylacetonate and **CH_3-Py** is γ-picoline was crystallized from ethanol. ^{19}F and ^{1}H NMR spectroscopy shows a dynamic equilibrium between the five possible octahedral diastereoisomers in solution[1].



Kubo-Sack formalism was used to fit experimental variable temperature and pressure ^{19}F and ^{1}H NMR spectra. The different routes of isomerisation were identified. Rate constants for all the isomerisation processes and for exchange with the free picoline were obtained and both energy and volume profiles are reported.

[1] G. Novitchi, F. Riblet, L.Helm, R. Scopelliti, A. Gulea, A. E. Merbach, *Magn.Reson.Chem*. 2004; **42**: 801-806

Analytical Chemistry **32**

Endocrine Disrupting Chemicals in Inhalable and Respirable Particulate Matter

Daniela Wenger[1,2], Andreas Gerecke[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), released from the technosphere into the atmosphere, are suspected to cause negative health effects in humans and wildlife as they interfere with the hormone system. Inhalation of EDCs might represent an important exposure pathway that should be considered in human risk assessment. However, the extent of exposure to endocrine disruptors in the air is largely unknown, yet.

The aim of this study is to provide insights into the exposure of humans and animals to EDCs from inhalable (PM_{10}) and respirable ($PM_{2.5}$) particulate matter. New analytical tools need to be developed to detect endocrine activity in 'air samples' (airborne particulate material, diesel particulate matter, indoor dust, cigarette particles). Our approach is to combine biological assays and chemical analysis via bioassay-directed fractionation of extracted samples. Luciferase reporter gene assays (CALUX) will be implemented to quantify the endocrine activity of (sub-) samples, mediated either by the human estrogen receptor (estrogenic compounds) or the aryl hydrocarbon receptor (TCDD-like compounds). Active substances in fractionated samples will be characterized by GC/HRMS and LC/MS/MS.

A preliminary test with particle extracts from bus filters revealed activity in the DR-CALUX bioassay due to TCDD-like compounds in diesel exhaust.

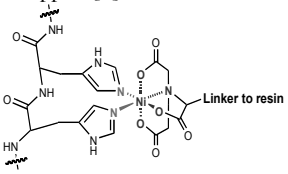
Analytical Chemistry **33**

Has the 6His-tag the optimal length?

Steven Knecht[1,2], Daniel Ricklin[1], Alex N. Eberle[2] and Beat Ernst[1]

[1] Institute of Molecular Pharmacy, Pharmacenter of the University of Basel, Klingelbergstrasse 50, CH-4056 Basel, Switzerland
[2] Laboratory of Endocrinology, Department of Research, University Hospital and University Children's Hospital, CH-4031, Basel, Switzerland

The work of Hochuli *et al.* [1] pioneered the efficient purification of recombinant proteins with tags, which typically consist of five to six consecutive histidine (His) residues attached to the *N*- or *C*-terminus of the protein. Its imidazole moieties can chelate the free coordination sites of metal ions, e.g. Ni^{2+}, which is itself immobilized as a chelate complex of nitrilotriacetic acid (NTA) bound to a solid support [2].



The interaction of Ni-NTA with 6His labeled proteins lies only in the micromolar range [3]. However, strong rebinding effects make the 6His-tag suitable for its application in affinity purification.
We investigated binding and rebinding effects of oligohistidines of various length using surface plasmon resonance. Affinity constants (K_Ds), and on- and off-rates (k_{on}, k_{off}) will be presented and discussed.

[1] E. Hochuli, W. Bannwarth, H. Döbelin, R. Gentz, D. Stüber, *Biotech.* **1988**, 1321-1325; E. Hochuli, H. Döbelin, A. Schacher, *J. Chromatogr.* **1987**, *411*, 177-184.
[2] F.H. Arnold, *Bio/Technology* **1991**, *9*, 151-156; J. Porath, J. Carlsson, I. Olsson, G. Belfrage, *Nature* **1975**, *258*, 598-599.
[3] L. Nieba *et al.*, *Anal. Biochem.* **1997**, *252*, 217-228

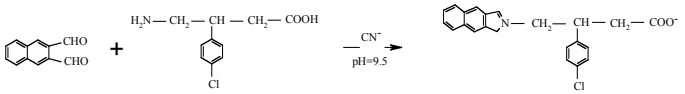
Analytical Chemistry **34**

Enantioseparation of baclofen by capillary electrophoresis with laser-induced fluorescence detection

Gamze Kavran Belin[1,2], Serge Rudaz[2], Jean-Luc Veuthey[2]

[1] Laboratory of Mass Spectrometry, University of Geneva, CH-1211, Geneva, Switzerland
[2] Laboratory of Pharmaceutical Analytical Chemistry, School of Pharmaceutical Sciences, EPGL, University of Geneva, CH-1211, Geneva, Switzerland

Capillary electrophoresis (CE) has become a powerful separation technique especially for the chiral analysis of drug substances. Baclofen is a muscle relaxant and an antispastic agent working directly in the brain and spinal cord [1] and is generally administered as a racemate. However, since the R-(-) enantiomer is more active and toxic than the S-(+) enantiomer, the chiral separation of baclofen in biological fluids is very important in order to achieve an optimal therapeutic drug monitoring (TDM). In order to enhance CE sensitivity, a laser induced fluorescence detector was selected and naphthalene-2,3-dicarboxaldehyde (NDA) was used for the derivatization of nonfluorescent baclofen.



NDA Baclofen Baclofen-NDA complex

Different highly sulfated cyclodextrin (HS-CD) such as α-, β- and γ-CD as well as the effects of organic modifiers, buffer concentration, nature and concentration of HS-CDs in the enantioseparation of racemic baclofen mixture by CE-LIF were investigated. With this sensitive method, developed electrophoretic conditions were finally applied to the analysis of baclofen enantiomers in human plasma.

[1] P.G. Loubser, N. M. Akman, *J. Pain Symptom Manage* **1996**, *12*, 241.

Analytical Chemistry **35**

Immobilized Trypsin Monolithic Bioreactors : On-line Digestion and Identification of Recombinant Proteins by LC-ESI-MS/MS

R. Nicoli[1], C. Stella[1], C. Temporini[2], E. Calleri[2], G. Massolini[2], S. Rudaz[1], J.-L. Veuthey[1]

[1]Laboratory of Pharmaceutical Analytical Chemistry - School of Pharmaceutical Sciences Geneva-Lausanne- University of Geneva - Switzerland
[2]Laboratory of Pharmaceutical Analytical Chemistry - University of Pavia - 27100 Pavia - Italy

Trypsin (EC 3.4.21.4) was covalently immobilized on different monolithic supports and resulting bioreactors used as immobilized enzyme reactors (IMERs) for on-line digestion, peptide separation and peptide mapping[1].
In order to determine the enzymatic activity, the substrate nα-benzoyl-L-arginine ethyl ester (BAEE) was selected and the production of nα-benzoyl-L-arginine UV-monitored at 254 nm. The apparent kinetic parameters (K_M* and V_{max}*) were also determined. The efficiency and the stability of the bioreactors were also evaluated with different recombinant proteins after on-line digestion.
The technique used for the separation and identification of peptides was high-performance liquid chromatography coupled with electrospray ionisation tandem mass spectrometry (LC-ESI-MS/MS).The number of matched peptides and the percentage of sequence coverage (Swissprot database) were used as comparison criteria for all bioreactors.

[1] Calleri, E.; Temporini, C.; Perani, E.; Stella, C.; Rudaz, S.; Lubda, D.; Mellerio, G.; Veuthey, J.-L.; Caccialanza, G.; Massolini, G., Journal of Chromatography, A (2004), 1045(1-2), 99-109.

Analytical Chemistry **36**

Determination of dissociation constants (K_d values) of protein-ligand complexes in solution using SUPREX

Sonal Mathur, Renato Zenobi*

Department of Chemistry and Applied Biosciences, Swiss Federal Institute of Technology, ETH Hoenggerberg, CH-8093 Zurich, Switzerland

Conventional methods for the quantitative analysis of protein-ligand binding interactions like calorimetric and spectroscopic techniques have several experimental limitations [1]. Mass spectrometry is a novel tool for studying biomolecular structures and noncovalent interactions. A new strategy based on H/D exchange and mass spectrometry, termed SUPREX (**S**tability of **U**npurified **P**roteins from **R**ates of H/D **Ex**change), to measure protein stability changes upon ligand binding was first proposed by Ghaemmaghami et al. [2]. This work is focussed on the determination of dissociation constants (K_d values) of noncovalent complexes using the SUPREX protocol. Our model system consists of the complex of angiogenin, a human recombinant protein and its inhibitor (N-65828) that selectively binds to the ribonucleolytic active site of angiogenin [3]. The advantages of SUPREX over the routinely used titration method include: measurements of picomole quantities of sample in high-throughput and automated fashion, and the ability to analyze either purified or unpurified protein-ligand complexes.

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[2] S. Ghaemmaghami, M. C. Fitzgerald, T. G. Oas, *Proc. Natl. Acad. Sci. U. S. A.* **2000**, *97*, 8296.
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Analytical Chemistry **37**

Evaluation of a 96-Well Method to Determine Aqueous Drug Solubility

Bruno Bard, Sophie Martel, Pierre-Alain Carrupt

LCT-Pharmacochimie, Section des sciences pharmaceutiques, Université de Genève, Quai Ernest-Ansermet 30, CH-1211, Genève 4, Suisse

Drug candidates are often poorly soluble in water, which results in unacceptably low drug absorption. Indeed, several experimental methods and computational models were developed with variable accuracy and complexity for the prediction of aqueous solubility in the early stage of drug development. Despite numerous efforts, accuracy obtained with computational methods remains currently too limited. Experimental methods for fast solubility determination are consequently highly desired.
A 96-wells UV detection approach already described by Millipore [1] was adapted as follows: a DMSO stock solution was diluted in aqueous buffer in a 96-well-filter plate to obtain a nominal compound concentration of 1000 μM. Because DMSO generally increases solubility of organic compounds compared to water solubility, evaluation of co-solvent effect on the solubility of a set of compounds was tested using different percentages of DMSO varying from 0.33 to 5%. These measurements were compared with values obtained by the potentiometric method (without DMSO), which is considered to be the standard method to determine kinetic solubility [2]. The results suggest a high compound dependent solubility behavior in presence of DMSO. But as a general result, decrease of DMSO percentage enhances the correlation between the two methods. At low fractions of co-solvent, solubility of the majority of compounds is poorly enhanced compared to potentiometric solubility values.

[1] Multiscreen Solubility filter plate protocole note, Millipore corp., **2003** Billerica.
[2] A. Avdeef, *Pharm. Pharmacol. Commun.* **1998**, 4, 165-178.

Analytical Chemistry **38**

Investigation of a new artificial membrane to mimic percutaneous absorption using PAMPA (Parallel Artificial Membrane Permeability Assay)

Giorgio Ottaviani, May-Claude Ryser, Sophie Martel, Pierre-Alain Carrupt

Ecole de Pharmacie Genève-Lausanne, LCT - Pharmacochimie, Section des sciences pharmaceutiques,Université de Genève, Quai Ernest-Ansermet 30, CH-1211 Genève 4, Suisse, http://www/unige.ch/pharmacochimie

The number of newly discovered and promising active compounds has increased dramatically in recent years, often matching poor pharmacokinetic properties, which are the rate-limiting step in drug research. Various predictive or experimental methods have been developed to assess drug permeation across biological membranes. The prediction of chemical transport across the skin is important for the optimization of topical and transdermal drug delivery [1].
Parallel artificial membrane permeability assay (PAMPA) is a high-throughput technique for measuring membrane permeability widely used in the pharmaceutical industry to predict oral passive absorption. The assay is carried out in 96-well microplates and the system consists of an artificial organic membrane separated by a donor and an acceptor compartment.
In this study, 2-nitrophenyloctylether (NPOE) was evaluated as artificial membrane, to predict passive percutaneous permeability in humans.
Permeation across the system has been studied for a range of compounds with diverse physicochemical properties. Kinetics measurements as well as pH permeability profiles were performed for a limited set of compounds to understand the influence of membrane retention and ionization state in penetration through NPOE liquid layers. Experimental permeability coefficients of neutral compounds were finally compared with in vitro permeability coefficients values through human skin taken from literature.

[1] J. J. Hostynek, R. S. Hinz, C. R. Lorence, M. Price, R. H. Guy, Crit. Rev. Tox. **1993**, 23, 171.

Analytical Chemistry **39**

Multivariate analysis of metabolomic data from *Arabidopsis thaliana* stressed by wounding with rapid LC/TOF-MS and gradient LC/quadrupole-MS analysis.

Julien Boccard[a,b], Elia Grata[a,c], Céline Didon[a], Aly Thiocone[a], Jean-Luc Wolfender[a], Serge Rudaz[c] and Pierre-Alain Carrupt[b]

Ecole de Pharmacie Genève-Lausanne, [a] Laboratoire de phytochimie et de pharmacognosie, [b] LCT - Pharmacochimie, [c] LACP-Méthodologie, Section des sciences pharmaceutiques,Université de Genève, Quai Ernest-Ansermet 30, CH-1211 Genève 4, Suisse, http://www.unige.ch/sciences/pharm/

Metabolomic strategies based on LC/MS have been developed in order to generate the most comprehensive survey of the metabolome modifications occurring in *A. thaliana* upon stress induction by wounding. LC/MS methods involving linear gradients on conventional column length with quadrupole instruments were compared to rapid methods on short columns with a high resolution time-of-flight (TOF) mass spectrometer. The methods were evaluated for their potential of discrimination between stressed (wounding by forceps) versus control *Arabidopsis* specimens, based on a global metabolome survey.
LC/MS data were first converted into filtered ion maps and compared thanks to an ANOVA test in order to statistically select discriminant masses. Selected ions were then processed through a vectorization procedure applied to the chromatographic and mass spectrometric information and further investigated by multivariate analysis. Principal Components Analysis (PCA) and Hierarchical Cluster Analysis (HCA) demonstrate a clear distinction between the sets of plant specimens. Furthermore, the specific detection of discrete induced metabolites is more easily achieved, selecting the highest discriminating m/z given by the overall data analysis.
Targeted analyses of the discrete metabolites involved in the stress phenomenon could then be performed. Such a strategy is applied to the detection of putative new low molecular mass regulators involved in defense signaling.

Analytical Chemistry **40**

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

Thomas Schmitz, Patrick Setz and Renato Zenobi

Department of Chemistry and Applied Biosciences, Swiss Federal Institute of Technology (ETH), Wolfgang-Pauli-Strasse 10, 8093 Zürich

Especially in materials science as well as in the biological sciences, there is great interest and need for spatially resolved chemical analysis (or imaging) on surfaces with nanometer resolution. We present a design that combines scanning near-field optical microscopy (SNOM) and laser ablation mass spectrometry (MS) that will allow molecular analysis with a spatial resolution of 50 - 150 nanometers to be performed at atmospheric pressure, which is not possible with any other method available today but crucial for applications in biology (i.e. analysis of living cells or tissue).

This 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. The first method by our group demonstrated nanoscale-MS at atmospheric pressure but had severe limitations in mass spectral performance [2].

We developed a combination of a highly sensitive ion trap and time-of-flight MS, including an optimized inlet and analyte transfer system from atmospheric pressure into the vacuum of the MS. We present laser ablation spectra of different samples, performed with different ionization methods developed for this specific purpose, including chemical ionization (CI) which is soft and particularly suited for fragile biological molecules.

[1] Kossakovski, D. A. et al. *Ultramicroscopy* **1998**, *71*, 111.
[2] Stöckle, R. et al. *Anal. Chem.* **2001**, *73*, 1399.

Analytical Chemistry **41**

Fundamental study on Aluminum and Calcium during LA-ICP-MS of glassy materials

Ivana Kroslakova and Detlef Günther

Laboratory of Inorganic Chemistry, Swiss Federal Institute of Technology, Wolfgang Pauli Strasse 10, 8093 Zürich, Switzerland

Fundamental processes (particles size formation and their ionization in the plasma) during Laser Ablation Inductively Coupled Plasma Mass Spectrometry measurements of the refractory elements Aluminum and Calcium were investigated in this study. Two the most frequently employed laser wavelengths for LA-ICP-MS (ArF 193 nm Excimer and Nd:YAG 266 nm) were used for generation of aerosol from SRM NIST 610.
Different particles size distributions and masses were sampled into the ion source by variation of laser sampling conditions (the wavelength, the ablation spot size), and Aluminum and Calcium ion intensities were measured. In addition, an implementation of an aerosol dilutor permitted modification of the specimen amount (while ablation conditions remained unchanged) sampled into the ICP. By means of dilution we were able to study the influence of the plasma load on the ionization behavior and the ionization efficiency of the elements investigated.
Reduction of sampled aerosol into the plasma by dilutor did not affect the measured Al/Ca intensity ratio for any of the investigated laser wavelength. On the other hand, the laser wavelength had significant influence on generated particles size distribution.
This study presents the influence of the plasma conditions on measured Al and Ca intensities and discusses the contribution of the examined experimental conditions on matrix effects in LA-ICP-MS.

Analytical Chemistry **42**

Characterization of a µs and ms Pulsed Glow Discharge

Daniel Fliegel and Detlef Günther

Laboratory of Inorganic Chemistry, Swiss Federal Institute of Technology, Wolfgang Pauli Strasse 10, 8093 Zürich, Switzerland

Pulsed glow discharges (GD) offer the advantage of a noble gas plasma environment with different transient ionization regimes [1]. The different ionization mechanisms are a result of the transient pulse profile. In the first hundred ns of one pulse primary electron impact ionization (EI) takes place. Later after pulse stabilization charge exchange (CE) and Penning ionization (PI) are the dominant processes and after the pulse PI is virtually the only ionization mechanism that occurs. Coupling an appropriate separation device like GC with the dynamic plasma and a detection system like MS or OES will result in real-time chemical speciation information about elemental, structural and parent molecular level of the compound of interest [2,3].
However the pulse power at given voltage or current is not independent from the source pressure. The maximum average pulse power that could be used without cooling of the cathode is dependent on the pulse frequency and the pulse duration. Without cooling of the cathode, the cathode material will be heated by the sputtering process until it will start to melt, if the average pulse power is chosen high enough [4]. The ratio between EI, CE and PI is strongly dependent on the applied source pressure and the selected power [5]. Therefore a careful characterization of the current-voltage profile is needed to predict the transient shifting of the ionization regime within one pulse cycle. This contribution discusses the pulse properties of µs and ms GD pulses at various source parameters.

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[3] Fliegel, D. et al. *Anal. Chem.* **2005**, *77*, 1847.
[4] Chapmann, B. '*Glow Discharge Processes'*, Wiley, New York (1980).
[5] Bogaerts, A. and Gijbels, R. *JAAS*, **2000**, *15*, 895.

Analytical Chemistry **43**

New strategies for the structural determination of induced compounds at the microgram level in a metabolomic study of *Arabidopsis thaliana*

A. Thiocone[1], G. Stupka[1], P. Eugster[1], E. Farmer[2], K. Hostettmann[1] and J.-L. Wolfender[1]

[1]Laboratoire de Pharmacognosie Phytochimie, Ecole de Pharmacie Genève-Lausanne, Université de Genève, CH-1211 Genève 4 [2]Departement de Biologie Moléculaire Végétale, Université de Lausanne, BB CH-1015 Lausanne

In plant metabolomic studies the amount of extract is often restricted and compounds responsible for key differences between plants sets might be found only in minute amounts. This is the case for the study of defence-induced compounds in the model plant *Arabidopsis thaliana* (Cruciferae) in which only a few rosette leaf are generally analysed in a given stress experiment. The detection of interesting key metabolome variations can be monitored by metabolite profiling with LC/MS, but the *de novo* characterisation of secondary metabolites of interest cannot rely on this approach alone in the absence of reference substances. The use of on-line complementary hyphenated techniques such as LC/NMR usually provides information on the main constituents of an extract but often fails to analyse efficiently minor constituents. Thus various strategies were developed off-line for the characterisation of small amount of metabolites collected at the microgram level based on a microfractionation triggered by LC/MS according to information provided by the metabolomic study. In this respect examples of datasets obtained on HPLC-microfractions with the combined use of a 5μl microflow capillary LC/NMR (CapNMR) , flow injection analysis TOF/ES-MS, ES-MS^n and GC/MS will be discussed.

Acknowledgements: Swiss National Science Foundation is thanked for supporting this work (grant 205320-107735 to JLW).

Analytical Chemistry **44**

Diffusion NMR using high-resolution heteronuclear experiments

Bruno Vitorge, Damien Jeannerat

Dep. of Org. Chem. University of Geneva, 30 Quai E. , 1211 Geneva 4, Switzerland

Diffusion measurements using pulse-field-gradient NMR (PFG-NMR) are the object of more and more interest in fields ranging from physical to bioorganic chemistry, and centered in organic synthesis and supramolecular chemistry.

In all but the simplest cases, proton NMR is not sufficient to resolve signals of the different components of complex mixtures. For example solution containing isomers, degradation products or any molecules undergoing slow chemical exchange.
When using standard diffusion experiments based on 1D proton spectra, one possibility consists in trying to determine diffusion rates of the overlapping components using multi-variable algorithms with the risks inherent to the determination of slightly differing diffusion rates. When sample concentration allows, a good alternative is to use the DOSY-HMQC experiment, the heteronuclear variant that benefit from the additional separation of signals according to carbon 13. But there also, nearly equivalent carbon chemical shift may not separate signals well enough to permit diffusion rate measurements. The reason may be that the carbon dimension would required a very large number of time increments which would make the acquisition time of the series of spectra unreasonably long, or because HMQC signals are intrinsically broad in the carbon dimension. The combination of spectral aliasing and HSQC type of experiments is the solution to both problems mentioned above. It allows to reach excellent signal resolution, a requisite for reliable diffusion rate measurements, in a drastically reduced total acquisition time.

Analytical Chemistry **45**

Investigation of High Molecular Weight Compounds in the Water-Soluble Fraction of Organic Urban Aerosols

Vera Samburova, Renato Zenobi, Markus Kalberer

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

In recent years, high molecular weight compounds have received considerable attention as components of atmospheric organic aerosols and cloud water constituents. Several studies showed that these compounds have characteristics similar to those of humic acids, especially their UV and fluorescence spectra. They are therefore often named humic-like substances (HULIS) [1, 2]. Size exclusion chromatography (SEC) was used to separate this class of aerosol components from lower molecular weight compounds [2], and various detection methods were used such as UV, IR, and fluorescence spectroscopy, as well as electrospray ionization mass spectrometry [2, 3]. However, their structure, chemical properties and abundance are still unknown.
In this study aerosol samples were collected in Zurich, Switzerland, at an urban background site and were analyzed for water-soluble organic compounds with high molecular weight using size exclusion chromatography (SEC) and laser/desorption ionization mass spectrometry (LDI-MS). Daily samples were collected during two campaigns in winter and summer, for one month each. Quantitative measurements, using humic and fulvic acid as surrogate standards, showed that 0.2-5.5 $\mu g/m^3$ of HULIS are present in the urban particles. Using polymethacrylic acid for size calibration, an upper mass limit of about 700 Da for HULIS in urban aerosols was estimated. These results are in good agreement with our LDI-MS measurements, which showed a mass distribution in the same range. During warmer periods it seems that generally enhanced photochemical activity increases the HULIS fraction in total OC, suggesting that secondary formation processes are important. In winter, with increased wood burning in Europe, a contribution of biomass burning to HULIS seems probable.

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Analytical Chemistry **46**

Plasma protein profiles after off-line protein precipitation or on-line Temperature Assisted Solid Phase Extraction (TASPE): Interest for protein matrix suppression evaluation in LC/MS analysis.

Emmanuel Bourgogne, Emmanuel Varesio and Gérard Hopfgartner

Life Sciences Mass Spectrometry, School of Pharmaceutical Sciences, University of Geneva, 20 Bvd d'Yvoy, 1211 Geneva 4, Switzerland

The performance of quantitative analysis of pharmaceuticals in biological fluids by LC-MS/MS can be strongly affected by matrix suppression. Suppression or enhancement of analyte signal during atmospheric pressure ionization is mainly caused by the co-elution of endogenous compounds. This results generally in poor precision and accuracy, requiring further method development. Therefore it becomes important to investigate in which extent matrix components like proteins, can affect the ionization process and how they are quantitatively and qualitatively removed during the sample preparation procedure.
The aim of this work is to compare remaining protein profiles by gel electrophoresis, after generic off-line protein precipitation or on-line solid phase extraction (SPE) approaches. For this purpose pooled plasma samples with EDTA or Li-Heparin anticoagulants were tested. Following the different sample procedures, the supernatant or SPE eluents were evaporated to dryness and reconstituted. After determination of the protein concentration using a Bradford assay, proteins were separated by SDS PAGE. For protein precipitation, acetonitrile, methanol, ethanol and perchloric acid were investigated. For SPE, a modified Prospekt 2 SPE device (Spark Holland) with different extraction cartridges chemistries was used. In this system, the extraction solvent can be heated up to 100°C to enhance protein wash-out.
Data on Li-heparin plasma showed that for acetonitrile proteins mostly below 60 kDa were remaining in the supernatant while for perchloric acid the reverse situation was observed. Comparison of various protein profiles will be presented for commonly used protein precipitation agents and for the different TASPE conditions.

Analytical Chemistry **47**

Thermal runaway in dry sewage sludge storage tanks: from molecular origins to technical measures of smoldering fire prevention

Martine Poffet and Titus A. Jenny*

University of Fribourg, Chemistry Department CH-1700 Fribourg, Switzerland

Until a few years ago sewage sludge was mainly used as fertilizer in agriculture. However the sewage sludge contains not only nutritive elements useful for plants, but also various different polluting substances and pathogenic agents what comes from the industries and the households. Since 2003 the use of the sewage sludge as a fertilizer is not allowed anymore [1]: ~200'000 tons of dried sewage sludge produced in Switzerland each year [2] have to be incinerated in respecting environmental norms [3].

In 2002, 19 % of the total amount of dried sewage sludge was transported by truck from the wastewater treatment plant to the cement industries for temporary storage and then incinerated. However troubles occur frequently during the storage of the dried sludge: auto-ignition and undeclared fires take place! Big pieces of burnt material have been removed from the storage tank since by burning the inorganic part of the dried sludge sinters together

Therefore to warrant a long-term use of dried sewage sludge as alternative fuels for the cement industries, its transport and its storage must be safe and secure. As these fires in the storage tanks disturb the working schedules and processes of the incineration plant from the cement industries and at the same time the timely elimination of the dried sludge from the wastewater treatment plant, a technical solution must be found as well as an explanation of the phenomena.

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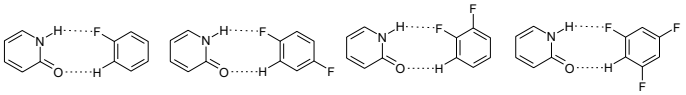
Analytical Chemistry **48**

Weak C-HO and N-HF Hydrogen Bonds: Dimers of 2-Pyridone with Fluorobenzenes

Roman Leist, Jann A. Frey and Samuel Leutwyler

Departement für Chemie und Biochemie, Universität Bern, Freiestrasse 3, CH-3012 Bern

Recent research in modified DNAs has shown that hydrophobic interactions can be exploited to build up DNA oligomers that combine aromatic, non-hydrogen-bonding nucleobase substitutes such as phenyl, biphenyl or perfluorophenyl groups [1,2]. These synthetic base pairs involve only weak C-HX (X=O, N) and X-HF hydrogen bonds. We investigated the hydrogen bond properties of isolated supersonically cooled dimers involving the C-HO and C-FH motifs.



The H-bonded complexes of 2-pyridone with 1-fluorobenzene (1FB), 1,2-difluorobenzene (1,2-DFB), 1,4-DFB, 1,3,5-trifluoro- and 1,2,4,5-tetrafluorobenzene were investigated by laser fluorescence and mass-selected S_1S_0 vibronic spectroscopies. Density functional (PW91, B3LYP) and correlated *ab initio* methods were employed to calculate the dimer structures and vibrations. The electronic binding energies of these dimers are D_e = 6-8 kcal/mol, supporting the observations [1,2] that the DNA oligomer stabilities in solution are due to entropic factors. The C-HO hydrogen bonds are stronger than the N-HF interactions. The H bond strength increases slightly with increasing F substitution.

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